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(54) Title: 56638, A NOVEL HUMAN NEPRILYSIN PROTEASE AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acids molecules, designated 56638 nucleic acid molecules, which encode novel neprilysin family members. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing 56638 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a 56638 gene has been introduced or disrupted. The invention still further provides isolated 56638 proteins, fusion proteins, antigenic peptides and anti-56638 antibodies. Diagnostic methods utilizing compositions of the invention are also provided. The invention also provides methods of modulating pain or pain related disorders utilizing the compositions of the invention. Accordingly, methods of treating, preventing and/or diagnosing pain or pain related disorders are disclosed.

## 56638, A Novel Human Neprilysin Protease and Uses Thereof

### Related Applications

This application claims priority to U.S. provisional application number 60/235,035 filed on September 25, 2000 and USSN 09/928,531, filed on August 13, 2001, the contents of which are incorporated herein by reference.

### Background of the Invention

Metalloproteases are a group of highly diverse, widely distributed proteolytic enzymes that depend on bound  $\text{Ca}^{2+}$  or  $\text{Zn}^{2+}$  for activity. Certain metalloproteases can readily utilize  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$  as well. About 30 families of metalloproteases are recognized, about half of which comprise enzymes containing the HEXXH motif (Rawlings et al. (1995) *Meth Enzymol* 248:183-228).

The M13 family of metalloproteases comprises neprilysin and neprilysin related proteases. Neprilysin is a mammalian enzyme that has the capacity to degrade enkephalins and other biologically active compounds. Other members of the neprilysin family of metalloproteases include endothelin-converting enzyme 1 (ECE-1), which processes the precursor of endothelin to release the active peptide; and Kell blood group glycoprotein, a major antigenic protein of erythrocytes. Typically, members of the neprilysin family have an N-terminal cytoplasmic domain, a transmembrane domain of about 23 amino acids, and an extracellular C-terminal domain (Rawlings et al. (1995) *Meth Enzymol* 248:183-228).

Biological functions of metalloproteases include pain modulation, protein maturation, degradation of proteins, such as extracellular matrix proteins, tumor growth, metastasis and angiogenesis. As such, they are likely to play important roles in a wide range of disorders including, but not limited to, pain, pain related disorders, inflammatory disorders, disorders of the trachea, the male reproductive system, or the hemotopoietic system. Accordingly, metalloproteases are a major target for drug action and development. Therefore, it is valuable to the field of pharmaceutical development to identify and characterize previously unknown metalloproteases.

### Summary of the Invention

The present invention is based, in part, on the discovery of a novel neprilysin family member, referred to herein as "56638." The nucleotide sequence of a cDNA encoding 56638 is shown in SEQ ID NO:1, and the amino acid sequence of a 56638 polypeptide is shown in  
5 SEQ ID NO:2. In addition, the nucleotide sequence of the coding region is depicted in SEQ ID NO:3.

Accordingly, in one aspect, the invention features a nucleic acid molecule which encodes a 56638 protein or polypeptide, e.g., a biologically active portion of a 56638 protein. In a preferred embodiment the isolated nucleic acid molecule encodes a polypeptide having  
10 the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention provides isolated 56638 nucleic acid molecules having the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3. In other embodiments, the  
15 invention provides a nucleic acid molecule which hybridizes under a stringent hybridization condition described herein to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or SEQ ID NO:3, wherein the nucleic acid encodes a full length 56638 protein or an active fragment thereof. In one embodiment, the protein fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ  
20 ID NO:2.

In a related aspect, the invention further provides nucleic acid constructs that include a 56638 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included, are vectors and host cells containing the 56638 nucleic acid  
25 molecules of the invention e.g., vectors and host cells suitable for producing 56638 nucleic acid molecules and polypeptides. The invention thus also provides vectors and host cells that express the 56294 metalloprotease nucleic acid molecules and polypeptides of the invention. Transgenic animals expressing 56638 metalloprotease nucleic acid molecules and polypeptides of the invention also are provided.

30 In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 56638-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to a 56638 encoding nucleic acid molecule are provided.

In a preferred embodiment, the 56638 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the  
5 56638 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 1000, 1500, 2000, 2500, 2600, 2700, 2721, 2750, 2800, 2900, or more contiguous nucleotides of SEQ ID NO:1 or 3. In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 56638 polypeptides.

10 In another embodiment, the invention provides 56638 polypeptides. Preferred polypeptides are 56638 proteins having a protease activity, e.g., a metalloprotease activity, e.g., a neprilysin-like peptidase activity, and preferably, a 56638 activity as described herein. In another aspect, the invention features, 56638 polypeptides, and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable to  
15 treatment and diagnosis of 56638 mediated or related disorders.

In other embodiments, the invention provides 56638 polypeptides, e.g., a 56638 polypeptide having the amino acid sequence shown in SEQ ID NO:2; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence  
20 which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid encodes a full length 56638 protein or an active fragment thereof. In one embodiment, the fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

25 In a preferred embodiment, the 56638 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 56638 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 200, 300, 400, 500, 600, 700, 750, 770, 771, 772, 773, 774, 775, 776, 777, 778, or more contiguous amino acids of SEQ ID NO:2. In one embodiment, the fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9  
30 contiguous residues from amino acids 1-9 of SEQ ID NO:2.



In a related aspect, the invention provides 56638 polypeptides or fragments operatively linked to non- 56638 polypeptides to form fusion proteins.

In another aspect, the invention provides methods of screening for agents, e.g., compounds, that modulate the expression or activity of the 56638 polypeptides or nucleic acids, e.g., compounds that modulate the normal pain response, aberrant or altered pain response, inflammatory response, fertility, spermatid activity, or erythroid cell activity.

In a preferred embodiment, the effect of an agent, e.g., a compound, on the pain response is evaluated by an analgesic test, e.g., the hot plate test, tail flick test, writhing test, paw pressure test, all electric stimulation test, tail withdrawal test, or formalin test.

10 In a preferred embodiment, the agent, e.g., compound, inhibits 56638 activity.

In a preferred embodiment, the agent, e.g., compound, increases endogenous levels of a 56638 substrate, e.g., enkephalin.

15 In still another aspect, the invention provides a process for modulating 56638 polypeptide or nucleic acid expression or activity, e.g. using the screened compounds. In certain embodiments, the methods involve treatment of conditions related to aberrant, e.g., decreased or increased expression of the 56638 polypeptides or nucleic acids, such as conditions involving pain response, aberrant or altered pain response, pain related disorders, inflammatory response, fertility, or hematopoietic cell activity.

20 In still another aspect, the invention features a method of modulating (e.g., enhancing or inhibiting) an activity of a cell (e.g., a neural, immune, e.g., erythroid, or reproductive cell), or a response (e.g., a pain, inflammatory, erythroid or reproductive response) in a subject. The method includes contacting the cell with, or administered to the subject, an agent, e.g., a compound, that modulates the activity or expression of a 56638 polypeptide or nucleic acid, in an amount effective to modulate the activity or the response.

25 In a preferred embodiment, the agent modulates (e.g., increases or decreases) metalloprotease activity, e.g., neprilysin-like peptidase activity.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 56638 nucleic acid by, e.g., modulating transcription, mRNA stability, etc.

30 In a preferred embodiment, the cell, e.g., the 56638-expressing cell, is a central or peripheral nervous system cell, e.g., a cell in an area involved in pain control, e.g., a cell in the substantia gelatinosa of the spinal cord, or a cell in the periaqueductal gray matter.

In other preferred embodiments, the cell, e.g., the 56638-expressing cell, is a cell of the male reproductive system, e.g., a spermatid cell. In those embodiments, the activity is a spermatid activity or fertility.

5 In other embodiments, the cell, e.g., the 56638-expressing cell, is a blood cell, e.g., an erythrocyte, erythroblast, or a B cell. In some embodiments, the methods involve treatment of conditions related to aberrant activity of a 56638-expressing cell, e.g., an erythroid cell, e.g., an erythrocyte or an erythroblast.

10 In a preferred embodiment, the agent, e.g., the compound, and the 56638-polypeptide or nucleic acid are contacted *in vitro* or *ex vivo*. In a preferred embodiment, the contacting step is effected *in vivo* in a subject, e.g., as part of a therapeutic or prophylactic protocol. The contacting or administering step(s) can be repeated.

15 Preferably, the subject is a human, e.g., a patient with pain or a pain-associated disorder disclosed herein. For example, the subject can be a patient with pain elicited from tissue injury, e.g., inflammation, infection, ischemia; pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches, e.g., migraine; pain associated with surgery; pain related to inflammation, e.g., irritable bowel syndrome; or chest pain. The subject can be a patient with complex regional pain syndrome (CRPS), reflex sympathetic dystrophy (RSD), causalgia, neuralgia, central pain and dysesthesia syndrome, carotidynia, neurogenic  
20 pain, refractory cervicobrachial pain syndrome, myofascial pain syndrome, craniomandibular pain dysfunction syndrome, chronic idiopathic pain syndrome, Costen's pain-dysfunction, acute chest pain syndrome, gynecologic pain syndrome, patellofemoral pain syndrome, anterior knee pain syndrome, recurrent abdominal pain in children, colic, low back pain syndrome, neuropathic pain, phantom pain from amputation, phantom tooth pain, or pain  
25 asymbolia. The subject can be a cancer patient, e.g., a patient with brain cancer, bone cancer, or prostate cancer. In other embodiments, the subject is a non-human animal, e.g., an experimental animal, e.g., an arthritic rat model of chronic pain, a chronic constriction injury (CCI) rat model of neuropathic pain, or a rat model of unilateral inflammatory pain by intraplantar injection of Freund's complete adjuvant (FCA).

30 In other embodiments, the subject is a human, e.g., a patient with infertility. The subject can be a cancer patient, e.g., a patient with prostate cancer. In yet other

embodiments, the subject is a non-human animal, e.g., an experimental animal, e.g., a rodent model for infertility.

In other embodiments, the subject is a human, e.g., a patient with erythroblastosis or anemia. The subject can be a cancer patient, e.g., a patient with common acute  
5 lymphoblastic leukemia.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof. The antibody can be conjugated to a therapeutic moiety selected from the group consisting of a cytotoxin, a cytotoxic agent and a radioactive metal ion.

10 In additional preferred embodiments, the agent is an antisense molecule, a ribozyme, a triple helix molecule, or a 56638 nucleic acid, or any combination thereof.

In a preferred embodiment, the agent is administered in combination with a cytotoxic agent.

In another aspect, the invention features a method of treating or preventing, in a  
15 subject, a 56638-associated disorder. The method includes administering to the subject, e.g., a subject at risk of, or afflicted with, a 56638-associated disorder, an agent, e.g., a compound as described herein, that modulates the activity or expression of a 56638 polypeptide or nucleic acid, in an amount effective to treat or prevent the disorder.

In a preferred embodiment, the disorder is pain or a pain related disorder.

20 In a preferred embodiment, the disorder is infertility or aberrant spermatid cell activity.

In a preferred embodiment, the disorder is anemia or erythroblastosis.

In a preferred embodiment, the disorder is a cancer, e.g., prostate cancer, bone cancer, or a lymphoblastic leukemia.

25 In a preferred embodiment, the subject is a subject as described herein, e.g., a human.

In still another aspect, the invention features a method for evaluating the efficacy of a treatment of a disorder, e.g., a disorder disclosed herein, in a subject. The method includes treating a subject with a protocol under evaluation; assessing the expression of a 56638 nucleic acid or 56638 polypeptide, such that a change in the level of 56638 nucleic acid or  
30 56638 polypeptide after treatment, relative to the level before treatment, is indicative of the efficacy of the treatment of the disorder.

In a preferred embodiment, the disorder is pain or a pain related disorder.

In a preferred embodiment, the disorder is infertility or aberrant spermatid cell activity.

In a preferred embodiment, the disorder is anemia or erythroblastosis.

5 In a preferred embodiment, the disorder is a cancer, e.g., prostate cancer, bone cancer, or a lymphoblastic leukemia.

In a preferred embodiment, the subject is a human.

In a preferred embodiment, the subject is an experimental animal, e.g., an animal model for an erythroid cell disorder, e.g., anemia, or erythroblastosis.

10 The invention also features a method of diagnosing a disorder, e.g., a disorder disclosed herein, in a subject. The method includes evaluating the expression or activity of a 56638 nucleic acid or a 56638 polypeptide, such that, a difference in the level of 56638 nucleic acid or 56638 polypeptide relative to a normal subject or a cohort of normal subjects is indicative of the disorder.

15 In a preferred embodiment, the disorder is pain or a pain related disorder.

In a preferred embodiment, the disorder is infertility or aberrant spermatid cell activity.

In a preferred embodiment, the disorder is an erythroid cell disorder, e.g., anemia or erythroblastosis.

20 In a preferred embodiment, the disorder is a cancer, e.g., prostate cancer, bone cancer, or a lymphoblastic leukemia.

In a preferred embodiment, the subject is a human.

In a preferred embodiment, the evaluating step occurs *in vitro* or *ex vivo*. For example, a sample, e.g., a blood sample, is obtained from the subject.

25 In a preferred embodiment, the evaluating step occurs *in vivo*. For example, by administering to the subject a detectably labeled agent that interacts with the 56638 nucleic acid or polypeptide, such that a signal is generated relative to the level of activity or expression of the 56638 nucleic acid or polypeptide.

30 The invention also provides assays for determining the activity of or the presence or absence of 56638 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

In further aspect, the invention provides assays for determining the presence or absence of a genetic alteration in a 56638 polypeptide or nucleic acid molecule, including for disease diagnosis.

5 In yet another aspect, the invention features a method for identifying an agent, e.g., a compound, which modulates the activity of a 56638 polypeptide, e.g., a 56638 polypeptide as described herein, or the expression of a 56638 nucleic acid, e.g., a 56638 nucleic acid as described herein, including contacting the 56638 polypeptide or nucleic acid with a test agent (e.g., a test compound); and determining the effect of the test compound on the activity of the 56638 polypeptide or nucleic acid to thereby identify a compound which modulates the  
10 activity of the 56638 polypeptide or nucleic acid.

In a preferred embodiment, the activity of the 56638 polypeptide is a metalloprotease activity, e.g., a neprilysin like peptidase activity, e.g., a neprilysin II-like activity.

In a preferred embodiment, the activity of the 56638 polypeptide is hydrolysis of a bioactive peptide, e.g., a neuropeptide, e.g., enkephalin.

15 In a preferred embodiment, the activity of the 56638 polypeptide is hydrolysis of a vasoactive peptide, e.g., endothelin.

In a preferred embodiment, the activity of the 56638 polypeptide is modulation of pain response.

20 In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or a 56638 nucleic acid, or any combination thereof.

In another aspect, the invention features a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g.,  
25 a nucleic acid or peptide sequence. At least one address of the plurality has a capture probe that recognizes a 56638 molecule. In one embodiment, the capture probe is a nucleic acid, e.g., a probe complementary to a 56638 nucleic acid sequence. In another embodiment, the capture probe is a polypeptide, e.g., an antibody specific for 56638 polypeptides. Also  
30 featured is a method of analyzing a sample by contacting the sample to the aforementioned array and detecting binding of the sample to the array.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

### Description of the Drawings

5        Figures 1A-1D show the 56638 cDNA sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2). The coding region is depicted as SEQ ID NO:3, and begins with an ATG.

Figure 2 is a hydropathy plot of 56638 metalloprotease. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The location of extracellular and intracellular loops is also indicated. Cysteine residues (cys) and N-glycosylation sites (Ngly) are indicated by short vertical lines just below the trace. The numbers corresponding to the amino acid sequence of human 56638 are indicated. Polypeptides of the invention include fragments which include: all or part of a hydrophobic sequence, i.e., a sequence above the dashed line, e.g., the sequence of 560-570 of SEQ ID NO:2; all or part of a hydrophilic sequence, i.e., a sequence below the dashed line, e.g., the sequence of 620-640 of SEQ ID NO:2; a sequence which includes a Cys or a glycosylation site.

Figure 3 depicts an alignment of the neprilysin signature sequence of human 56638 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper sequence is the consensus amino acid sequence (SEQ ID NO:4), while the lower amino acid sequence corresponds to amino acids 572-778 of SEQ ID NO:2.

Figure 4 is a bar graph depicting relative 56638 mRNA expression as determined by TaqMan assays on mRNA derived from the human tissues: adrenal gland, brain, heart, kidney, liver, lung, mammary gland, placenta, prostate, salivary gland, muscle, small intestine, spleen, stomach, teste, thymus, trachea, uterus, spinal cord, skin, and dorsal root ganglion. The highest 56638 mRNA expression was observed in spinal cord, dorsal root ganglion (DRG), small intestine, testes, and trachea.

### Detailed Description

The human 56638 sequence (SEQ ID NO:1), which is approximately 2953

nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 2340 nucleotides, including the stop codon (SEQ ID NO:3).

Although the ATG at position 1-3 of SEQ ID NO:3 is the preferred start site of translation, other embodiments are included wherein, e.g., the ATG at position 28-30 of SEQ ID NO:3 is the start site of translation. The coding sequence encodes an 779 amino acid protein (SEQ ID NO:2). The human 56638 protein of SEQ ID NO:2 is predicted to have a signal peptide at about amino acid 1-44 of SEQ ID NO:2.

Human 56638 sequence contains the following regions or other structural features: an M13 peptidase (neprilysin) domain (PF01431) from about amino acid 572 to 778 of SEQ ID NO:2, which includes the characteristic HEXXH zinc-binding active site of metallopeptidases (PS00142) located at about amino acid 610 to 619 of SEQ ID NO:2.

The human 56638 sequence can additionally include: eight N-glycosylation sites (PS00001) located from about amino acid 156 to 159, from about amino acid 177 to 180, from about amino acid 207 to 210, from about amino acid 243 to 246, from about amino acid 350 to 353, from about amino acid 530 to 533, from about amino acid 638 to 641, and from about amino acid 657 to 660 of SEQ ID NO:2;

one cAMP and cGMP-dependent protein kinase phosphorylation site (PS00004) from about amino acid 183 to 186 of SEQ ID NO:2;

eleven protein kinase C phosphorylation sites (PS00005) from about amino acid 158 to 160, from about amino acid 244 to 246, from about amino acid 269 to 271, from about amino acid 361 to 363, from about amino acid 391 to 393, from about amino acid 412 to 414, from about amino acid 493 to 495, from about amino acid 503 to 505, from about amino acid 551 to 553, from about amino acid 726 to 728, and from about amino acid 735 to 737 of SEQ ID NO:2;

eight casein kinase II phosphorylation sites (PS00006) from about amino acid 137 to 140, from about amino acid 158 to 161, from about amino acid 179 to 182, from about amino acid 429 to 432, from about amino acid 445 to 448, from about amino acid 482 to 485, from about amino acid 503 to 506, and from about amino acid 673 to 676 of SEQ ID NO:2;

three tyrosine kinase phosphorylation sites (PS00007) from about amino acid 435 to 442, from about amino acid 520 to 526, and from about amino acid 645 to 653 of SEQ ID NO:2;

nine N-myristoylation sites (PS00008) from about amino acid 9 to 14, from about amino acid 44 to 49, from about amino acid 78 to 83, from about amino acid 93 to 98, from about amino acid 547 to 552, from about amino acid 608 to 613, from about amino acid 683 to 688, from about amino acid 706 to 711, and from about amino acid 750 to 755 of SEQ ID NO:2;

a prenyl group binding site (CAAX box) (PS00294) from about amino acid 776 to 779 of SEQ ID NO:2; and

a signal peptide from about amino acid 1 to 44 of SEQ ID NO:2, resulting in a mature protein of 822 amino acids, from amino acid 45 to 779 of SEQ ID NO:2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

A plasmid containing the nucleotide sequence encoding human 56638 (clone "Fbh566338FL") was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 56638 protein contains a significant number of structural characteristics in common with members of the neprilysin family of metalloproteases. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

The neprilysin family comprises a number of related enzymes that share high structural homology and a common catalytic mechanism that involves cleavage of a protein



substrate by hydrolysis of an amide bond that depends upon the presence of a metal ion, e.g., zinc. Neprilysins are mammalian membrane metalloproteases which contain the active site consensus sequence VxxHExxH (SEQ ID NO:5; amino acids 610 to 619 of SEQ ID NO:2) found in other zinc metalloproteases. The histidines are two of the three Zn-coordinating ligands, and the glutamate plays a role in catalysis by polarizing a water molecule. The hydrolysis occurs through the formation of a pentacoordinated complex of the metal which includes the three Zn-coordinating amino acids of the peptidase, the oxygen of the scissile bond, and the water molecule that is initially bound to the Zn atom. For a review, see, Barrett (1995) *Methods in Enzymol* 248:263-283. In addition, neprilysin family members share other structural features. They can be highly glycosylated type II integral membrane proteins, and they can have a cluster of conserved cysteine residues following the transmembrane domain which are involved in stabilizing the active enzyme through the formation of sulfide bridges (Tanja et al (2000) *Biochem Biophys Res Comm* 271:565-570).

The human 56638 proteins of the present invention show significant homology to members of the neprilysin metallopeptidase family, and in particular, to the mouse NL1/SEP and the rat neprilysin II proteins (Ghaddar et al. (2000) *Biochem J* 347:419-429; Ikeda et al. (1999) *J Biol Chem* 274:32469-32477). Like mouse NL1/SEP and rat neprilysin II, 56638 is a secreted protein. 56638 has the characteristic VxxHExxH (SEQ ID NO:5) zinc-binding metallopeptidase consensus sequence (PS00142), located at about amino acid 610 to 619 of SEQ ID NO:2. Neprilysin family members include neprilysin, endothelin converting enzyme (ECE), Kell Blood group antigen, PEX, and X-converting enzyme (XCE), and soluble secreted endopeptidase (SSE). Examples of substrates of the neprilysin peptidase family include, but are not limited to, neuropeptides involved in pain control, e.g., enkephalin, somatostatin, and substance P; and vasoactive peptides that mediate inflammation and pain, e.g., neurotensin, atrial natriuretic peptide (ANP), neurokinin, tachykinin, bradykinin, and endothelin (Checler et al. (1983) *J Neurochem* 41:375; Matsas et al. (1983) *Proc Natl Acad Sci USA* 80:3111; Matsas et al. (1984) *Biochem J* 223:433; Stephenson and Kenny (1987) *Biochem J* 241:237; Turner and Tazawa (1997) *FASEB J* 11:355-364). Mouse NL1/Sep has been shown to cleave enkephalin *in vivo*. Enkephalin, a major substrate of neprilysin, is one of several naturally occurring morphinelike substances released from nerve endings of the central nervous system and the adrenal medulla. It acts as an analgesic and sedative in the

body and appears to affect mood and motivation. As neprilysin is responsible for the inactivation of enkephalin and other bioactive peptides involved in inflammation and pain, neprilysins are critical for the proper function of many physiological systems, including neurotransmission, pain control, inflammatory response, and vascular tone.

5 Other neprilysin family members include a marker of common acute lymphoblastic leukemia antigen present at the surface of B cells (Roques et al. (1993) *Pharmacol Rev* 45:87), and the Kell blood group antigen (Lee et al. (1999) *Proc Natl Acad Sci USA* 88:6353-6357). Kell antigens are highly immunogenic and may cause severe fetal anemia in sensitized mothers, erythroblastosis in newborn infants, and severe hemolytic reactions if  
10 mismatched blood is transfused.

A 56638 polypeptide can include a "neprilysin domain" or regions homologous with a "neprilysin domain." A 56638 polypeptide can optionally further include a signal peptide; at least one, two, three, four, five, six, seven, preferably eight N-glycosylation sites; at least one cAMP and cGMP-dependent protein kinase phosphorylation site; at least one, two, three,  
15 four, five, six, seven, eight, nine, ten, preferably eleven, protein kinase C phosphorylation sites; at least one, two, three, four, five, six, seven, preferably eight, casein kinase II phosphorylation sites; at least one, two, preferably three, tyrosine kinase phosphorylation sites; at least one, two, three, four, five, six, seven, eight, preferably nine, N-myristoylation sites; at least one prenyl group binding site.

20 As used herein, the term "neprilysin domain" includes an amino acid sequence of about 50 to 350 amino acid residues in length, more preferably about 100 to 300 amino acid residues, or about 200 to 215 amino acids, and having a bit score for the alignment of the sequence to the neprilysin domain (HMM) of at least 100, preferably 150, more preferably 200, most preferably 250 or more. Preferably, the domain includes a zinc-binding active site  
25 of metallopeptidase domains (PS00142) located at about amino acid 610 to 619 of SEQ ID NO:2. The neprilysin domain (HMM) has been assigned the PFAM Accession Number PF01431 (<http://genome.wustl.edu/Pfam/.html>). An alignment of the neprilysin domain (amino acids 572 to 778 of SEQ ID NO:2) of human 56638 with a consensus amino acid sequence derived from a hidden Markov model derived from PFAM is depicted in  
30 Figure 3.

In a preferred embodiment 56638 polypeptide or protein has a "neprilysin domain" or

a region which includes at least about 50 to 350, more preferably about 100 to 300, or 200 to 215 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "neprilysin," e.g., the neprilysin domain of human 56638 (e.g., residues 572 to 778 of SEQ ID NO:2).

5           To identify the presence of a "neprilysin" domain in a 56638 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters  
([http://www.sanger.ac.uk/Software/Pfam/HMM\\_search](http://www.sanger.ac.uk/Software/Pfam/HMM_search)). For example, the hmmsf program,  
10       which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer et al. (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in  
15       Gribskov et al.(1990) *Meth Enzymol* 183:146-159; Gribskov et al.(1987) *Proc Natl Acad Sci USA* 84:4355-4358; Krogh et al.(1994) *J Mol Biol.* 235:1501-1531; and Stultz et al. (1993) *Protein Sci* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of a "neprilysin" domain in the amino acid sequence of human 56638 at about residues 572 to 778 of SEQ ID  
20       NO:2 (see Figure 1).

          A 56638 protein can further include a signal peptide, and is predicted to be a secreted protein. As used herein, a "signal peptide" or "signal sequence" refers to a peptide of about 20 to 60, preferably about 30 to 50, more preferably, about 44 amino acid residues in length which occurs at the N-terminus of secretory and integral membrane proteins and which  
25       contains a majority of hydrophobic amino acid residues. For example, a signal sequence contains at least about 20 to 60, preferably about 30 to 50, more preferably, 44 amino acid residues, and has at least about 40-70%, preferably about 50-65%, and more preferably about 55-60% hydrophobic amino acid residues (e.g., alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, or proline). Such a "signal sequence", also referred to in  
30       the art as a "signal peptide," serves to direct a protein containing such a sequence to a lipid bilayer. For example, in one embodiment, a 56638 protein contains a signal sequence of

about amino acids 1 to 44 of SEQ ID NO:2. The "signal sequence" is cleaved during processing of the mature protein. The mature 56638 protein corresponds to amino acids 45 to 778 of SEQ ID NO:2.

As used herein, a "56638 activity," "biological activity of 56638," or "functional activity of 56638," refers to an activity exerted by a 56638 protein, polypeptide or nucleic acid molecule on e.g., a 56638-responsive cell or on a 56638 substrate, e.g., a protein substrate, as determined *in vivo* or *in vitro*. In one embodiment, a 56638 activity is a direct activity, such as an association with a 56638 target molecule. A "target molecule" "substrate" or "binding partner" is a molecule with which a 56638 protein binds or interacts in nature. A 56638 activity can also be an indirect activity, e.g., a cellular signaling activity mediated by interaction of the 56638 protein with a 56638 binding partner. In an exemplary embodiment, 56638 is an enzyme for an enkephalin substrate.

Based on the above-described sequence similarities and the tissue distribution described below, the 56638 molecules of the present invention are predicted to have similar biological activities as neprilysin metalloprotease family members. Thus, in accordance with the invention, a 56638 metalloprotease or subsequence or variant polypeptide may have one or more domains and, therefore, one or more activities or functions characteristic of a neprilysin metalloprotease family member, including, but not limited to, (1) the ability to modulate the activity of a bioactive peptide, (2) the ability to cleave a neprilysin substrate, e.g., enkephalin, (3) the ability to modulate pain or inflammation response, (4) the ability to modulate spermatid cell activity or infertility, or (5) the ability to modulate hematopoietic cell activity, e.g., erythroid cell activity or B cell activity. Thus, the 56638 molecules can act as novel diagnostic targets and therapeutic agents for controlling neprilysin associated disorders.

Neprilysin is involved in the inactivation of the opioid enkephalins in the brain, which induce analgesic responses. Inhibitors of neprilysin are thus able to potentiate the analgesic effects of exogenous enkephalins, as evaluated by analgesic tests on animals, e.g., the hot plate test, tail flick test, writhing test, paw pressure test, all electric stimulation test, tail withdrawal test, or formalin test (Roques et al. (1995) *Methods in Enzymology* 248:263-283). Thus, 56638 neprilysin or subsequence or variant having neprilysin activity is capable of cleaving one or more protein substrates, e.g., biologically active neuropeptides, e.g.,

enkephalin, substance P, or somatostatin, to modulate pain response.

Neprilysin family members are also involved in the inflammatory response. Besides enkephalin, other neprilysin substrates include endothelin (a polypeptide produced by endothelial cells that stimulates contraction of the underlying smooth muscle of blood vessel walls), and vasoactive peptides that cause vasodilation and pain, e.g., neurotensin, atrial natriuretic peptide (ANP), neurokinin, tachykinin, bradykinin, and endothelin.

56638 mRNA is expressed in human adrenal gland, brain, heart, kidney, liver, lung, mammary gland, placenta, prostate, salivary gland, muscle, small intestine, spleen, stomach, testes, thymus, trachea, uterus, spinal cord, skin, and dorsal root ganglion (DRG). The highest 56638 mRNA expression was observed in testes, trachea, brain, spinal cord and DRG (Figure 4).

As 56638 mRNA is highly expressed in human testis, it suggests a role for 56638 in, e.g., fertility or spermatid development. Human 56638 appears to be a human orthologue of mouse neprilysin NL1/SEP and the rat neprilysin II proteins (Ghaddar et al. (2000) *Biochem J* 347:419-429; Tanja et al. (2000) *Biochem Biophys Res Comm* 271:565-570). Like 56638, mouse NL1/SEP and rat neprilysin II are highly expressed in testis, and are secreted proteins. The rat and mouse proteins have been localized to the seminiferous tubules and, specifically, to spermatids (*Ibid*). Testicular neprilysin enzymes may act to modulate enkephalins acting as intratesticular paracrine/autocrine factors. Thus, the 56638 molecules can act as novel diagnostic targets and therapeutic agents controlling sperm formation or other processes related to fertility, e.g., spermatogenesis or fertilization.

Other examples of disorders involving the testis and epididymis include, but are not limited to, congenital anomalies such as cryptorchidism, regressive changes such as atrophy, inflammations such as nonspecific epididymitis and orchitis, granulomatous (autoimmune) orchitis, and specific inflammations including, but not limited to, gonorrhea, mumps, tuberculosis, and syphilis, vascular disturbances including torsion, testicular tumors including germ cell tumors that include, but are not limited to, seminoma, spermatocytic seminoma, embryonal carcinoma, yolk sac tumor choriocarcinoma, teratoma, and mixed tumors, tumor of sex cord-gonadal stroma including, but not limited to, Leydig (interstitial) cell tumors and sertoli cell tumors (androblastoma), and testicular lymphoma, and miscellaneous lesions of tunica vaginalis.

As 56638 mRNA is highly expressed in human trachea, it also suggests a role for 56638 in modulation of the activity of bioactive peptides in the trachea, bronchus, and lung. Thus, the 56638 molecules can act as novel diagnostic targets and therapeutic agents controlling respiratory disorders, e.g., chronic obstructive pulmonary disease, emphysema, amyloidosis, lung disease, lung cancer, sleep apnea, bronchitis, pneumonias, silicosis, pulmonary edema, interstitial restrictive lung diseases, pulmonary embolus, or pulmonary hypertension.

56638 mRNA is also highly and widely expressed in the central and peripheral nervous system. More specifically, high levels of 56638 mRNA expression were found in human brain, spinal cord and DRG (Figure 4). Taqman experiments in rat showed that 56638 is expressed in pituitary gland, spinal cord, brain, nerve, TRG, and DRG. *In situ* hybridization with a 56638 probe shows that 56638 is heterogeneously expressed in monkey CNS, including expression in cerebral cortex, spinal cord, brain stem nucleus and hypothalamus. Hence, 56638 is likely a neuropeptidase, e.g., a neuropeptidase involved in pain response.

Animal models of pain response include, but are not limited to, axotomy, the cutting or severing of an axon; chronic constriction injury (CCI), a model of neuropathic pain which involves ligation of the sciatic nerve in rodents, e.g., rats; or intraplantar Freund's adjuvant injection as a model of arthritic pain. Other animal models of pain response are described in, e.g., ILAR Journal (1999) Volume 40, Number 3 (entire issue). Taqman experiments on rodent models of pain response showed that the 56638 gene is up-regulated in DRG seven days after axotomy and seven days after CCI. *In situ* hybridization experiments in rat pain models show up-regulation of the 56638 gene one and seven days after axotomy and after complete Freund's adjuvant intraplantar injection. These levels go back to normal at later time points. No contralateral effects were observed. These experiments indicate a role for the 56638 molecule in pain response.

Therefore, neprilysin and 56638 associated disorders can detrimentally affect regulation and modulation of the pain response; and vasoconstriction, inflammatory response and pain therefrom. Examples of neprilysin associated disorders in which the 56638 molecules of the invention may be directly or indirectly involved include pain, pain syndromes, and inflammatory disorders, including inflammatory pain.

As the 56638 polypeptides of the invention may modulate 56638-mediated activities, they may be useful for developing novel diagnostic and therapeutic agents for 56638-mediated or related disorders. For example, the 56638 molecules can act as novel diagnostic targets and therapeutic agents controlling pain, pain disorders, and inflammatory disorders.

5 For example, a 56638 inhibitor can be useful in the treatment of pain, as 56638 inhibition could increase the endogenous levels of enkephalins and thereby increase the associated analgesic response.

Examples of pain conditions include, but are not limited to, pain elicited during various forms of tissue injury, e.g., inflammation, infection, and ischemia; pain associated  
10 with musculoskeletal disorders, e.g., joint pain, or arthritis; tooth pain; headaches, e.g., migraine; pain associated with surgery; pain related to inflammation, e.g., irritable bowel syndrome; chest pain; or hyperalgesia, e.g., excessive sensitivity to pain (described in, for example, Fields (1987) Pain, New York:McGraw-Hill). Other examples of pain disorders or pain syndromes include, but are not limited to, complex regional pain syndrome (CRPS),  
15 reflex sympathetic dystrophy (RSD), causalgia, neuralgia, central pain and dysesthesia syndrome, carotidynia, neurogenic pain, refractory cervicobrachial pain syndrome, myofascial pain syndrome, craniomandibular pain dysfunction syndrome, chronic idiopathic pain syndrome, Costen's pain-dysfunction, acute chest pain syndrome, nonulcer dyspepsia, interstitial cystitis, gynecologic pain syndrome, patellofemoral pain syndrome, anterior knee  
20 pain syndrome, recurrent abdominal pain in children, colic, low back pain syndrome, neuropathic pain, phantom pain from amputation, phantom tooth pain, or pain asymbolia (the inability to feel pain). Other examples of pain conditions include pain induced by parturition, or post partum pain.

Agents that modulate 56638 polypeptide or nucleic acid activity or expression can be  
25 used to treat pain elicited by any medical condition. A subject receiving the treatment can be additionally treated with a second agent, e.g., an anti-inflammatory agent, an antibiotic, or a chemotherapeutic agent, to further ameliorate the condition.

The 56638 molecules can also act as novel diagnostic targets and therapeutic agents controlling pain caused by other disorders, e.g., cancer, e.g., prostate cancer. For example,  
30 endothelin, which is inactivated by neprilysin, is associated with the excruciating, debilitating pain that comes when prostate cancer invades the bone (reviewed in Nelson and Carducci

(2000) *BJU Int* 85 Suppl 2:45-8). In addition, a neprolysin family member can be a marker of common acute lymphoblastic leukemia antigen present at the surface of B cells (Roques et al. (1993) *Pharmacol Rev* 45:87). Accordingly, the 56638 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular proliferative and/or differentiative disorders, or pain therefrom.

The 56638 molecules can also act as novel diagnostic targets and therapeutic agents for brain disorders. Disorders involving the brain include, but are not limited to, disorders involving neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects, forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply, intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoencephalitis, including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2, *Varicella-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination;



degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem, including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including striatonigral  
5 degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as  
10 leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B<sub>1</sub>) deficiency and vitamin B<sub>12</sub> deficiency, neurologic sequelae of  
15 metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephalopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma,  
20 oligodendroglioma, and ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor  
25 (malignant schwannoma), and neurocutaneous syndromes (phakomatoses), including neurofibromatosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

Examples of such cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, or metastatic disorders. A metastatic tumor can arise from a  
30 multitude of primary tumor types, including but not limited to those of lung, prostate, colon, breast, and liver origin.

As used herein, the terms "cancer", "hyperproliferative", and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or  
5 may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic  
10 hyperproliferative cells include proliferation of cells associated with wound repair.

The terms "cancer" or "neoplasms" include malignancies of the various organ systems, such as those affecting lung, breast, thyroid, lymphoid, gastrointestinal, and the genito-urinary tract. The terms "cancer" or "neoplasms" also includes adenocarcinomas that include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer  
15 and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine, and cancer of the esophagus.

The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas,  
20 prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon, and ovary. The term also includes carcinosarcomas, e.g., malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.  
25 The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

Additional examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic  
30 disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias, e.g., erythroblastic

leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T- cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Sternberg disease.

In addition, a neprolysin family member can be a Kell blood group antigen (Lee et al. (1999) *Proc Natl Acad Sci USA* 88:6353-6357). Kell antigens are highly immunogenic and may cause severe fetal anemia in sensitized mothers, erythroblastosis in newborn infants, and severe hemolytic reactions if mismatched blood is transfused. Therefore, the 56638 molecules can also act as novel diagnostic targets and therapeutic agents controlling disorders related to hematopoietic cells, e.g., blood cell- (e.g., erythroid-) associated disorders, e.g., anemia, or erythroblastosis.

As used herein, the term "erythroid associated disorders" include disorders involving aberrant (increased or deficient) erythroblast proliferation, e.g., an erythroleukemia, and aberrant (increased or deficient) erythroblast differentiation, e.g., an anemia. Erythrocyte-associated disorders include anemias such as, for example, drug- (chemotherapy-) induced anemias, hemolytic anemias due to hereditary cell membrane abnormalities, such as hereditary spherocytosis, hereditary elliptocytosis, and hereditary pyropoikilocytosis; hemolytic anemias due to acquired cell membrane defects, such as paroxysmal nocturnal hemoglobinuria and spur cell anemia; hemolytic anemias caused by antibody reactions, for example to the RBC antigens, or antigens of the ABO system, Lewis system, Ii system, Rh system, Kidd system, Duffy system, and Kell system; methemoglobinemia; a failure of erythropoiesis, for example, as a result of aplastic anemia, pure red cell aplasia, myelodysplastic syndromes, sideroblastic anemias, and congenital dyserythropoietic anemia; secondary anemia in non-hematologic disorders, for example, as a result of chemotherapy,

alcoholism, or liver disease; anemia of chronic disease, such as chronic renal failure; and endocrine deficiency diseases.

The 56638 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of immune disorders. Examples of hematopoietic disorders or diseases include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

The 56638 protein, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 thereof are collectively referred to as "polypeptides or proteins of the invention" or "56638 polypeptides or proteins." Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "56638 nucleic acids." 56638 molecules refer to 56638 nucleic acids, polypeptides, and antibodies.

As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes

nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived.

5 For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when  
10 produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current*  
15 *Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the  
20 temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high  
25 stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under a stringency condition described herein to the sequence of SEQ ID NO:1 or SEQ ID NO:3,  
30 corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or

DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 56638 protein, preferably a mammalian 56638 protein, and can further include non-coding regulatory sequences, and introns.

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of 56638 protein having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-56638 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-56638 chemicals. When the 56638 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 56638 (e.g., the sequence of SEQ ID NO:1 or 3) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present in the neprilysin domain, are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side

chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 56638 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 56638 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 56638 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or SEQ ID NO:3, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of a 56638 protein includes a fragment of a 56638 protein which participates in an interaction between a 56638 molecule and a non-56638 molecule. Biologically active portions of a 56638 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 56638 protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include fewer amino acids than the full length 56638 proteins, and exhibit at least one activity of a 56638 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 56638 protein, e.g., neprilysin activity e.g., the ability to cleave a bioactive peptide. A biologically active portion of a 56638 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a 56638 protein can be used as targets for developing agents which modulate a 56638 mediated activity, e.g., a neprilysin activity.

Particularly preferred 56638 polypeptides of the present invention have an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:2. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:2 are termed substantially identical.

In the context of nucleotide sequence, the term "substantially identical" is used herein

to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:1 or 3 are termed substantially identical.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 20%, 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second sequence to the 56638 amino acid sequence of SEQ ID NO:2 having 206 amino acid residues (the neprilysin domain), at least 40, 60, preferably at least 80, more preferably at least 100, even more preferably at least 120, and even more preferably at least 140, 160, or 180 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment,



the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J Mol Biol* (48):444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) *CABIOS* 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul et al. (1990) *J Mol Biol* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 56638 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 56638 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

"Misexpression or aberrant expression," as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type

in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Subject," as used herein, can refer to a mammal, e.g., a human, or to an experimental or animal or disease model, e.g., a rodent model of pain, e.g., an arthritic rat, a CCI rodent, or an axotomized rodent. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal.

A "purified preparation of cells," as used herein, refers to, in the case of plant or animal cells, an *in vitro* preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

#### Isolated Nucleic Acid Molecules

In one aspect, the invention provides, an isolated or purified, nucleic acid molecule that encodes a 56638 polypeptide described herein, e.g., a full length 56638 protein or a fragment thereof, e.g., a biologically active portion of 56638 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify a nucleic acid molecule encoding a polypeptide of the invention, 56638 mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, or a portion of the nucleotide sequence. In one embodiment, the nucleic acid molecule includes sequences encoding the human 56638

protein (i.e., "the coding region" of SEQ ID NO:1, as shown in SEQ ID NO:3), as well as 5' untranslated sequences. Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., SEQ ID NO:3) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to a fragment of the protein from about amino acid 572 to 778. In another embodiment, the fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 3, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about: 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or a portion, preferably of the same length, of any of these nucleotide sequences.

#### 56638 Nucleic Acid Fragments

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of a 56638 protein, e.g., an immunogenic or biologically active portion of a 56638 protein. A fragment can comprise those nucleotides of SEQ ID NO:1, which encode a neprilysin domain of human 56638. The nucleotide sequence determined from the cloning of the 56638 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 56638 family members, or fragments thereof, as well as 56638 homologues, or fragments thereof, from other species.

In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding

region. Other embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. In one embodiment, the fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

5 Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 100 amino acids in length. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not be construed as encompassing those fragments that may have been disclosed prior to the  
10 invention.

A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment can also include one or more domain, region, or functional site described herein. Thus, for example, a 56638 nucleic acid fragment can include a sequence corresponding to a neprilysin domain. In one embodiment,  
15 the fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

56638 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably  
20 about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1 or SEQ ID NO:3, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or SEQ ID NO:3.

In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should  
25 be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid  
30 which encodes the neprilysin domain of 56638 (from amino acids 572 to 778 of SEQ ID NO:2).

In another embodiment a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of a 56638 sequence, e.g., a domain, region, site or other sequence described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differ by one base from a sequence disclosed herein or from a naturally occurring variant. For example, primers suitable for amplifying all or a portion of any of the following regions are provided: a neprilysin domain from about amino acid 572 to 778 of SEQ ID NO:2.

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

A nucleic acid fragment encoding a "biologically active portion of a 56638 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, which encodes a polypeptide having a 56638 biological activity (e.g., the biological activities of the 56638 proteins are described herein), expressing the encoded portion of the 56638 protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 56638 protein. For example, a nucleic acid fragment encoding a biologically active portion of 56638 includes a neprilysin domain, e.g., amino acid residues about 572 to 778 of SEQ ID NO:2. A nucleic acid fragment encoding a biologically active portion of a 56638 polypeptide, may comprise a nucleotide sequence which is greater than 300 or more nucleotides in length.

In a preferred embodiment, a nucleic acid fragment includes a nucleotide sequence comprising nucleotides SEQ ID NO:1 or SEQ ID NO:3, or a portion thereof, wherein each portion is about 50 or longer nucleotides and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:3.

In preferred embodiments, a nucleic acid fragment includes a nucleotide sequence which is about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2721, 2730, 2740, 2750, 2760, 2770, 2780, 2790, 2800, 2850, 2900, 2950 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:3. In one embodiment, the fragment includes at least 1, preferably 2, 3, 6, 9, 15, 30, 45, 90, 120, 160, 200, or 230, or more, contiguous nucleotides

from nucleotides 1-232 of SEQ ID NO:1. In another embodiment, the fragment includes at least 1, preferably 2, 3, 6, 9, 15, 30, 45, 90, 120, 160, 200, or more, contiguous nucleotides from nucleotides 1-1910, 2195-2212 or 2900-2953 of SEQ ID NO:1.

In a preferred embodiment, a nucleic acid fragment has a nucleotide sequence other than (e.g., differs by at least one, two, three, five, ten or more nucleotides from) the nucleotide sequence of GenBank Accession Nos. AX033274, AW955378, AW991607 or AW373239. E.g., a nucleic acid fragment can: include one or more nucleotides from SEQ ID NO: 1 or SEQ ID NO:3 outside the region of nucleotides 2444-2953 of SEQ ID NO:1; not include all of the nucleotides of AX033274, AW955378, AW991607 or AW373239, e.g., can be one or more nucleotides shorter (at one or both ends) than the sequence of AX033274, AW955378, AW991607 or AW373239; or can differ by one or more nucleotides in the region of overlap.

#### 56638 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 56638 proteins as those encoded by the nucleotide sequence disclosed herein). In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the invention can be chosen for having codons, which are preferred, or non preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques,

including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

5           In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO: 1 or 3, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

10           Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95%, 95-99%, or more identical to the nucleotide sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can  
15           readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 2 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 56638 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 56638 gene.

20           Preferred variants include those that are correlated with neprilysin activity.

          Allelic variants of 56638, e.g., human 56638, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 56638 protein within a population that maintain the ability to bind substrates and hydrolyze them. Functional allelic variants will typically contain only conservative  
25           substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 56638, e.g., human 56638, protein within a population that do not have the ability to bind and/or hydrolyze a substrate. Non-functional allelic variants will typically contain a non-conservative  
30           substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion, or deletion in critical residues or critical regions of

the protein.

Moreover, nucleic acid molecules encoding other 56638 family members and, thus, which have a nucleotide sequence which differs from the 56638 sequences of SEQ ID NO:1 or SEQ ID NO:3 are intended to be within the scope of the invention.

5 *Antisense Nucleic Acid Molecules, Ribozymes and Modified 56638 Nucleic Acid Molecules*

In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to 56638. An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA  
10 sequence. The antisense nucleic acid can be complementary to an entire 56638 coding strand, or to only a portion thereof (e.g., the coding region of human 56638 corresponding to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 56638 (e.g., the 5' and 3' untranslated regions).

15 An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 56638 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 56638 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 56638 mRNA, e.g., between the -10 and +10 regions of the target gene  
20 nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized  
25 using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an  
30 antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an



antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 56638 protein to thereby

inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An alpha-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids Res* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for a 56638-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 56638 cDNA disclosed herein (i.e., SEQ ID NO:1 or SEQ ID NO:3), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 56638-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, 56638 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a

pool of RNA molecules. See, e.g., Bartel and Szostak (1993) *Science* 261:1411-1418.

56638 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 56638 (e.g., the 56638 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 56638 gene in target cells. See generally, Helene (1991) *Anticancer Drug Des* 6(6):569-84; Helene et al. (1992) *Ann NY Acad Sci* 660:27-36; and Maher (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

15 A 56638 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup et al. (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996) *supra*; Perry-O'Keefe et al. *Proc Natl Acad Sci*. 93: 14670-675.

PNAs of 56638 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 56638 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1

nucleases (Hyrup (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup et al. (1996) *supra*; Perry-O'Keefe, *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport  
5 across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc Natl Acad Sci USA* 86:6553-6556; Lemaitre et al. (1987) *Proc Natl Acad Sci USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon  
10 (1988) *Pharm Res* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a 56638 nucleic acid of the  
15 invention, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantitating the presence of the 56638 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

#### 20 Isolated 56638 Polypeptides

In another aspect, the invention features, an isolated 56638 protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-56638 antibodies. 56638 protein can be isolated from cells or tissue sources using standard protein purification techniques. 56638 protein or fragments thereof  
25 can be produced by recombinant DNA techniques or synthesized chemically. In one embodiment, the fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and  
30 alternative translational and post-translational events. The polypeptide can be expressed in

systems, e.g., cultured cells, which result in substantially the same post-translational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of post-translational modifications, e.g., glycosylation or cleavage, present when expressed in a native cell.

5           In a preferred embodiment, a 56638 polypeptide has one or more of the following characteristics:

- (i) it has the ability to hydrolyze a peptide substrate;
- (ii) it has the ability to bind zinc;
- (iii) it has the ability to modulate the activity of a bioactive peptide;
- 10       (iv) it has the ability to modulate pain response;
- (v) it has an overall sequence similarity of at least 60%, preferably at least 70, 75, 80, 90, or 95%, with a polypeptide of SEQ ID NO:2;
- (vi) it has a neprolysin signature sequence which is preferably about 70%, 80%, 90% or 95% homologous with amino acid residues from about amino acids 659-865 of SEQ ID
- 15       NO:2, or a fragment thereof (e.g., amino acid residues 659-700, 700-750, 750-800, or 800-865), and a zinc protease zinc-binding region signature which is preferably about 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more, homologous with amino acid residues from about amino acid 572-778 of SEQ ID NO:2, or a fragment thereof (e.g., amino acid residues 572-600, 600-650, 650-700, 700-750, or 750-778 of SEQ ID NO:2).

20           In a preferred embodiment, the 56638 protein, or fragment thereof, includes one or more of amino acids 1, 2, 3, 4, 5, 6, 7, 8, 9, from amino acids 1-9 of SEQ ID NO:2. Preferably, the 56638 protein, or fragment thereof, includes amino acids 1-9 of SEQ ID NO:2.

25           In a preferred embodiment the 56638 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID NO:2. In one embodiment it differs by about 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acids. Preferred embodiments include fragments wherein the sequence differs by about 1, 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from about amino acids 1-9 of SEQ ID NO:2. In another it differs by at least one but less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in SEQ ID NO:2

30       by at least 1 %. In another it differs from the corresponding sequence in SEQ ID NO:2 by at least one residue but less than 20%, 15%, 10%, 5%, or 1% of the residues in it differ from the

corresponding sequence in SEQ ID NO:2. If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. The differences are, preferably, differences or changes at a non essential residue or a conservative substitution. In  
5 a preferred embodiment the differences are not in the neprilysin domain (about amino acids 572 to 778 of SEQ ID NO:2). In another preferred embodiment one or more differences are in the neprilysin domain (about amino acids 572 to 778 of SEQ ID NO:2).

Other embodiments include a protein that contains one or more changes in amino acid sequence, e.g., a change in an amino acid residue which is not essential for activity, e.g., a  
10 change in one or more of amino acids 1, 2, 3, 4, 5, 6, 7, 8, or 9 of SEQ ID NO:2. Such 56638 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity.

In one embodiment, the protein includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or more homologous to SEQ ID NO:2.

15 A 56638 protein or fragment is provided which varies from the sequence of SEQ ID NO:2 in regions defined by amino acids about 1 to 571 and 779 by at least one but by less than 15, 10 or 5 amino acid residues in the protein or fragment but which does not differ from SEQ ID NO:2 in regions defined by amino acids about 572 to 778 of SEQ ID NO:2. If this comparison requires alignment the sequences should be aligned for maximum homology.  
20 "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. In some embodiments the difference is at a non essential residue or is a conservative substitution, while in others the difference is at an essential residue or is a non conservative substitution.

In one embodiment, a biologically active portion of a 56638 protein includes a  
25 neprilysin domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 56638 protein.

In a preferred embodiment, the 56638 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 56638 protein is substantially identical to  
30 SEQ ID NO:2. In yet another embodiment, the 56638 protein is substantially identical to SEQ ID NO:2 and retains the functional activity of the protein of SEQ ID NO:2, as described

in detail in the subsections above. In another preferred embodiment, the 56638 protein has an amino acid sequence including at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 contiguous amino acid residues from about amino acid 1-9 of SEQ ID NO:2.

56638 Chimeric or Fusion Proteins

5           In another aspect, the invention provides 56638 chimeric or fusion proteins. As used herein, a 56638 "chimeric protein" or "fusion protein" includes a 56638 polypeptide linked to a non-56638 polypeptide. A "non-56638 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 56638 protein, e.g., a protein which is different from the 56638 protein and which is derived  
10       from the same or a different organism. The 56638 polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of a 56638 amino acid sequence. In a preferred embodiment, a 56638 fusion protein includes at least one (or two) biologically active portion of a 56638 protein. The non-56638 polypeptide can be fused to the N-terminus or C-terminus of the 56638 polypeptide.

15           The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST-56638 fusion protein in which the 56638 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 56638. Alternatively, the fusion protein can be a 56638 protein containing a heterologous signal sequence at its N-terminus. In certain host  
20       cells (e.g., mammalian host cells), expression and/or secretion of 56638 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, e.g., an IgG constant region, or human serum albumin.

25           The 56638 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 56638 fusion proteins can be used to affect the bioavailability of a 56638 substrate. 56638 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 56638 protein; (ii) mis-regulation of the 56638 gene; and (iii) aberrant post-translational modification of a 56638 protein.

30           Moreover, the 56638-fusion proteins of the invention can be used as immunogens to

produce anti-56638 antibodies in a subject, to purify 56638 ligands and in screening assays to identify molecules which inhibit the interaction of 56638 with a 56638 substrate.

Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A 56638-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 56638 protein.

#### Variants of 56638 Proteins

In another aspect, the invention also features a variant of a 56638 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the 56638 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of a 56638 protein. An agonist of the 56638 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 56638 protein. An antagonist of a 56638 protein can inhibit one or more of the activities of the naturally occurring form of the 56638 protein by, for example, competitively modulating a 56638-mediated activity of a 56638 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 56638 protein.

Variants of a 56638 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a 56638 protein for agonist or antagonist activity.

Libraries of fragments e.g., N-terminal, C-terminal, or internal fragments, of a 56638 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 56638 protein. In one embodiment, a fragment includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property are known in the art. Recursive ensemble mutagenesis (REM), a new

technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 56638 variants (Arkin and Yourvan (1992) *Proc Natl Acad Sci USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

5           Cell based assays can be exploited to analyze a variegated 56638 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, which ordinarily responds to 56638 in a substrate-dependent manner. The transfected cells are then contacted with 56638 and the effect of the expression of the mutant on signaling by the 56638 substrate can be detected, e.g., by measuring neprilysin peptidase activity. Plasmid  
10       DNA can then be recovered from the cells that score for inhibition, or alternatively, potentiation of signaling by the 56638 substrate, and the individual clones further characterized.

          In another aspect, the invention features a method of making a 56638 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of  
15       a naturally occurring 56638 polypeptide, e.g., a naturally occurring 56638 polypeptide. The method includes: altering the sequence of a 56638 polypeptide, e.g., altering the sequence by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

          In another aspect, the invention features a method of making a fragment or analog of  
20       a 56638 polypeptide a biological activity of a naturally occurring 56638 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of a 56638 polypeptide, e.g., altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

#### 25       Anti-56638 Antibodies

          In another aspect, the invention provides an anti-56638 antibody, or a fragment thereof (e.g., an antigen-binding fragment thereof). The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. As used herein, the term "antibody" refers to a protein comprising  
30       at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as



VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDR's has been precisely defined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917, which are incorporated herein by reference). Each VH and VL is composed of three CDR's and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The anti-56638 antibody can further include a heavy and light chain constant region, to thereby form a heavy and light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are interconnected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 KDa or 214 amino acids) are encoded by a variable region gene at the NH<sub>2</sub>-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin "heavy chains" (about 50 KDa or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to the antigen, e.g., 56638 polypeptide or fragment thereof. Examples of antigen-binding fragments of the anti-56638 antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The anti-56638 antibody can be a polyclonal or a monoclonal antibody. In other embodiments, the antibody can be recombinantly produced, e.g., produced by phage display or by combinatorial methods.

Phage display and combinatorial methods for generating anti-56638 antibodies are known in the art (as described in, e.g., Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* International Publication No. WO 92/18619; Dower *et al.* International Publication No. WO 91/17271; Winter *et al.* International Publication WO 92/20791; Markland *et al.* International Publication No. WO 92/15679; Breitling *et al.* International Publication WO 93/01288; McCafferty *et al.* International Publication No. WO 92/01047; Garrard *et al.* International Publication No. WO 92/09690; Ladner *et al.* International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO*

*J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-4137; and Barbas et al. (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference  
5 herein).

In one embodiment, the anti-56638 antibody is a fully human antibody (e.g., an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, e.g., a rodent (mouse or rat), goat, primate (e.g., monkey), camel antibody. Preferably, the non-human antibody is a  
10 rodent (mouse or rat antibody). Method of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g.,  
15 Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuailon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).  
20

An anti-56638 antibody can be one in which the variable region, or a portion thereof, e.g., the CDR's, are generated in a non-human organism, e.g., a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibodies generated in a non-human organism, e.g., a rat or mouse, and then modified, e.g., in the variable framework  
25 or constant region, to decrease antigenicity in a human are within the invention.

Chimeric antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent  
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Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).

A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDR's (of heavy and or light immunoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDR's may be replaced with non-human CDR's. It is only necessary to replace the number of CDR's required for binding of the humanized antibody to a 56638 or a fragment thereof. Preferably, the donor will be a rodent antibody, e.g., a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDR's is called the "donor" and the immunoglobulin providing the framework is called the "acceptor." In one embodiment, the donor immunoglobulin is a non-human (e.g., rodent). The acceptor framework is a naturally-occurring (e.g., a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

An antibody can be humanized by methods known in the art. Humanized antibodies can be generated by replacing sequences of the Fv variable region which are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby

incorporated by reference. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against a 56638 polypeptide or fragment thereof. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Humanized or CDR-grafted antibodies can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDR's of an immunoglobulin chain can be replaced. See e.g., U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference.

Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. Preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a humanized antibody will have framework residues identical to the donor framework residue or to another amino acid other than the recipient framework residue. To generate such antibodies, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Preferred locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., US 5,585,089). Criteria for selecting amino acids from the donor are described in US 5,585,089, e.g., columns 12-16 of US 5,585,089, the e.g., columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on December 23, 1992.

A full-length 56638 protein or, antigenic peptide fragment of 56638 can be used as an immunogen or can be used to identify anti-56638 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of 56638 should

include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of 56638. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. In a preferred embodiment, the antigenic peptide includes at least 1, preferably 2, 3, 4, 5, 6, 7, 8, or 9 contiguous residues from amino acids 1-9 of SEQ ID NO:2.

Fragments of 56638 which include residues about 620-640 of SEQ ID NO:2 can be used to make, e.g., used as immunogens or used to characterize the specificity of an antibody, antibodies against hydrophilic regions of the 56638 protein. Similarly, a fragment of 56638 which includes residues about 560-570 of SEQ ID NO:2 can be used to make an antibody against a hydrophobic region of the 56638 protein; a fragment of 56638 which include residues about 572 to 778 of SEQ ID NO:2, or a portion thereof (e.g., amino acid residues 572-600, 600-650, 650-700, 700-750, or 750-778 of SEQ ID NO:2), about can be used to make an antibody against the neprilysin region of the 56638 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

Preferred epitopes encompassed by the antigenic peptide are regions of 56638 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 56638 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 56638 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In a preferred embodiment the antibody binds an epitope on any domain or region on 56638 proteins described herein.

Chimeric, humanized, but most preferably, completely human antibodies are desirable for applications which include repeated administration, e.g., therapeutic treatment (and some diagnostic applications) of human patients.

The anti-56638 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher et al. (1999) *Ann N Y Acad Sci* 880:263-80; and (1996) *Reiter Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for

different epitopes of the same target 56638 protein.

In a preferred embodiment the antibody has effector function and/or can fix complement. In other embodiments the antibody does not recruit effector cells; or fix complement.

5 In a preferred embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example, it is a isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, e.g., it has a mutagenized or deleted Fc receptor binding region.

10 An anti-56638 antibody (e.g., monoclonal antibody) can be used to isolate 56638 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-56638 antibody can be used to detect 56638 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-56638 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment  
15 regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labelling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase;  
20 examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable  
25 radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

The invention also includes a nucleic acid that encodes an anti-56638 antibody, e.g., an anti-56638 antibody described herein. Also included are vectors which include the nucleic acid and cells transformed with the nucleic acid, particularly cells which are useful for producing an antibody, e.g., mammalian cells, e.g. CHO or lymphatic cells.

30 The invention also includes cell lines, e.g., hybridomas, which make an anti-56638 antibody, e.g., and antibody described herein, and method of using said cells to make a 56638

antibody.

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

A vector can include a nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., proteins, mutant forms of proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded



therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic  
5 cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New  
10 England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in 56638 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 56638  
15 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

To maximize recombinant protein expression in *E. coli* is to express the protein in a  
20 host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al. (1992)  
25 *Nucleic Acids Res* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The 56638 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in  
mammalian cells.

30 When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived

from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of  
5 suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the  
10 neurofilament promoter; Byrne and Ruddle (1989) *Proc Natl Acad Sci USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science*  
15 249:374-379) and the alpha-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic  
20 acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub et al. (1986) *Antisense RNA as a molecular tool for genetic analysis*, Reviews  
25 - Trends in Genetics, Vol. 1(1).

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a 56638 nucleic acid molecule within a recombinant expression vector or a 56638 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell"  
30 and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain

modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

5 A host cell can be any prokaryotic or eukaryotic cell. For example, a 56638 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are  
10 intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

A host cell of the invention can be used to produce (i.e., express) a 56638 protein. Accordingly, the invention further provides methods for producing a 56638 protein using the  
15 host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 56638 protein has been introduced) in a suitable medium such that a 56638 protein is produced. In another embodiment, the method further includes isolating a 56638 protein from the medium or the host cell.

20 In another aspect, the invention features, a cell or purified preparation of cells which include a 56638 transgene, or which otherwise misexpress 56638. The cell preparation can consist of human or non human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a 56638 transgene, e.g., a heterologous form of a 56638, e.g., a gene derived from humans (in the case of a non-human  
25 cell). The 56638 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous 56638, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed 56638 alleles or for use in drug screening.

30 In another aspect, the invention features, a human cell, e.g., a CNS cell, transformed with nucleic acid that encodes a subject 56638 polypeptide.

Also provided are cells, preferably human cells, e.g., human hematopoietic or fibroblast cells, in which an endogenous 56638 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 56638 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous 56638 gene. For example, an endogenous 56638 gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published May 16, 1991.

#### Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a 56638 protein and for identifying and/or evaluating modulators of 56638 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 56638 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a

56638 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a 56638 transgene in its genome and/or expression of 56638 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 56638 protein can further be bred to other transgenic animals carrying other transgenes.

56638 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed, e.g., below.

#### Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express a 56638 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect a 56638 mRNA (e.g., in a biological sample) or a genetic alteration in a 56638 gene, and to modulate 56638 activity, as described further below. The 56638 proteins can be used to treat disorders characterized by insufficient or excessive production of a 56638 substrate or production of 56638 inhibitors. In addition, the 56638 proteins can be used to screen for naturally occurring 56638 substrates, to screen for drugs or compounds which modulate 56638 activity, as well as to treat disorders characterized by insufficient or excessive production of 56638 protein or production of 56638 protein forms which have decreased, aberrant or unwanted activity compared to 56638 wild type protein (e.g., pain or pain related disorders, and inflammatory disorders). Moreover, the anti-56638 antibodies of the invention can be used to detect and isolate 56638 proteins, regulate the bioavailability of 56638 proteins, and modulate 56638 activity.

A method of evaluating a compound for the ability to interact with, e.g., bind, a subject 56638 polypeptide is provided. The method includes: contacting the compound with the subject 56638 polypeptide; and evaluating ability of the compound to interact with, e.g., to bind, form a complex with, or act as a substrate for, or of, the subject 56638 polypeptide.

5 This method can be performed *in vitro*, e.g., in a cell free system, or *in vivo*, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with subject 56638 polypeptide. It can also be used to find natural or synthetic inhibitors of subject 56638 polypeptide. Screening methods are discussed in more detail below.

#### 10 Screening Assays

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to 56638 proteins, have a stimulatory or inhibitory effect on, for example, 56638 expression or 56638 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 15 56638 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., 56638 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

20 In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 56638 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 56638 protein or polypeptide or a biologically active portion thereof.

25 The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckermann et al. (1994) *J Med Chem* 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries;

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synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc Natl Acad Sci USA* 90:6909; Erb et al. (1994) *Proc Natl Acad Sci USA* 91:11422; Zuckermann et al. (1994) *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew Chem Int Ed Engl.* 33:2059; Carell et al. (1994) *Angew Chem Int Ed Engl* 33:2061; and in Gallop et al. (1994) *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull et al. (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla et al. (1990) *Proc Natl Acad Sci USA* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner, *supra*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 56638 protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate 56638 activity is determined. Determining the ability of the test compound to modulate 56638 activity can be accomplished by monitoring, for example, neprilysin activity, e.g., zinc binding activity or hydrolysis of a peptide substrate, e.g., enkephalin cleavage activity. The cell, for example, can be of mammalian origin, e.g., human.

The ability of the test compound to modulate 56638 binding to a compound, e.g., a 56638 substrate, or to bind to 56638 can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to 56638 can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, 56638 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 56638 binding to a 56638 substrate in a complex. For example, compounds (e.g.,

56638 substrates) can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting.

Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by  
5 determination of conversion of an appropriate substrate to product.

The ability of a compound (e.g., a 56638 substrate) to interact with 56638 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with 56638 without the labeling of either the compound or the 56638. McConnell et al. (1992) *Science*  
10 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 56638.

In yet another embodiment, a cell-free assay is provided in which a 56638 protein or  
15 biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 56638 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the 56638 proteins to be used in assays of the present invention include fragments that participate in interactions with non-56638 molecules, e.g., fragments with high surface probability scores.

20 Soluble and/or membrane-bound forms of isolated proteins (e.g., 56638 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®,  
25 Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and  
30 the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.



The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz et al., U.S. Patent No. 5,631,169; Stavrianopoulos et al., U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment, determining the ability of the 56638 protein to bind to a target molecule or substrate can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander and Urbaniczky (1991) *Anal Chem* 63:2338-2345 and Szabo et al. (1995) *Curr Opin Struct Biol* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either 56638, an anti-56638 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to

a 56638 protein, or interaction of a 56638 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain  
5 that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/56638 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test  
10 compound and either the non-adsorbed target protein or 56638 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 56638 binding or activity  
15 determined using standard techniques.

Other techniques for immobilizing either a 56638 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 56638 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and  
20 immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored  
25 on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn,  
30 can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 56638

protein or target molecules but which do not interfere with binding of the 56638 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 56638 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 56638 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 56638 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas and Minton (1993) *Trends Biochem Sci* 18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard (1998) *J Mol Recognit* 11(1-6):141-8; Hage and Tweed (1997) *J Chromatogr B Biomed Sci Appl* 699(1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the 56638 protein or biologically active portion thereof with a known compound which binds 56638 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 56638 protein, wherein determining the ability of the test compound to interact with a 56638 protein includes determining the ability of the test compound to preferentially bind to 56638 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules

such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 56638 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a 56638 protein through modulation of the activity of a downstream effector of a 56638 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, e.g., by

competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been  
5 formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an  
10 immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain  
15 immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled  
20 with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components,  
25 and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

30 In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or

extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 56638 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J Biol Chem* 268:12046-12054; Bartel et al. (1993) *Biotechniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 56638 ("56638-binding proteins" or "56638-bp") and are involved in 56638 activity. Such 56638-bps can be activators or inhibitors of signals by the 56638 proteins or 56638 targets as, for example, downstream elements of a 56638-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 56638 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively 56638 protein can be the fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a 56638-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., lacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 56638 protein.

In another embodiment, modulators of 56638 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of

56638 mRNA or protein evaluated relative to the level of expression of 56638 mRNA or protein in the absence of the candidate compound. When expression of 56638 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 56638 mRNA or protein expression.

5 Alternatively, when expression of 56638 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 56638 mRNA or protein expression. The level of 56638 mRNA or protein expression can be determined by methods described herein for detecting 56638 mRNA or protein.

10 In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, e.g., a peptidase assay, and the ability of the agent to modulate the activity of a 56638 protein can be confirmed *in vivo*, e.g., in an animal such as an animal model for pain response, e.g., an arthritic rodent, a CCI rodent model for neuropathic pain, or  
15 a rodent with induced unilateral inflammatory pain by intraplantar injection of Freund's complete adjuvant.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., a 56638 modulating agent, an antisense 56638 nucleic  
20 acid molecule, a 56638-specific antibody, or a 56638-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

#### Detection Assays

25 Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 56638 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological  
30 sample. These applications are described in the subsections below.

### Chromosome Mapping

The 56638 nucleotide sequences or portions thereof can be used to map the location of the 56638 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the 56638 sequences with genes associated with disease.

Briefly, 56638 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 56638 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the 56638 sequences will yield an amplified fragment.

A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human chromosomes. (D'Eustachio et al. (1983) *Science* 220:919-924).

Other mapping strategies e.g., *in situ* hybridization (described in Fan et al. (1990) *Proc Natl Acad Sci USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 56638 to a chromosomal location.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross



hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in McKusick, Mendelian Inheritance in Man, available on-line  
5 through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland et al. (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and  
10 unaffected with a disease associated with the 56638 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome  
15 spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

#### Tissue Typing

56638 sequences can be used to identify individuals from biological samples using,  
20 e.g., restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

25 Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 56638 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences  
30 from individuals, prepared in this manner, can provide unique individual identifications, as

each individual will have a unique set of such DNA sequences due to allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 56638 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

#### Use of Partial 56638 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

The 56638 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 56638 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., 56638 primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

#### Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes 56638.

Such disorders include, e.g., disorders associated with the misexpression of 56638 gene: pain, pain related disorders, inflammatory disorders, disorders of the trachea, the male reproductive system, or the hemotopoietic system.

The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 56638 gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 56638 gene;

detecting, in a tissue of the subject, the misexpression of the 56638 gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA;

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of a 56638 polypeptide.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 56638 gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more

nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1, or naturally occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the 56638 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 56638 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 56638.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of a 56638 gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 56638 protein or a nucleic acid, which hybridizes specifically with the gene. There and other embodiments are discussed below.

#### Diagnostic and Prognostic Assays

Diagnostic and prognostic assays of the invention include method for assessing the expression level of 56638 molecules and for identifying variations and mutations in the sequence of 56638 molecules.

##### Expression Monitoring and Profiling.

The presence, level, or absence of 56638 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 56638 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 56638 protein such that the presence of 56638 protein or nucleic acid is detected in the biological sample. The term "biological

sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 56638 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 56638 genes; measuring the amount of protein encoded by the 56638 genes; or measuring the activity of the protein encoded by the 56638 genes.

The level of mRNA corresponding to the 56638 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 56638 nucleic acid, such as the nucleic acid of SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 56638 mRNA or genomic DNA. The probe can be disposed on an address of an array, e.g., an array described below. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array described below. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 56638 genes.

The level of mRNA in a sample that is encoded by one of 56638 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis (1987) U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.*, (1989), *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, (1988) *Bio/Technology* 6:1197), rolling

circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 56638 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 56638 mRNA, or genomic DNA, and comparing the presence of 56638 mRNA or genomic DNA in the control sample with the presence of 56638 mRNA or genomic DNA in the test sample. In still another embodiment, serial analysis of gene expression, as described in U.S. Patent No. 5,695,937, is used to detect 56638 transcript levels.

A variety of methods can be used to determine the level of protein encoded by 56638. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect 56638 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 56638 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence,

enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 56638 protein include introducing into a subject a labeled anti-56638 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In  
5 another embodiment, the sample is labeled, e.g., biotinylated and then contacted to the antibody, e.g., an anti-56638 antibody positioned on an antibody array (as described below). The sample can be detected, e.g., with avidin coupled to a fluorescent label.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 56638 protein, and comparing the presence of  
10 56638 protein in the control sample with the presence of 56638 protein in the test sample.

The invention also includes kits for detecting the presence of 56638 in a biological sample. For example, the kit can include a compound or agent capable of detecting 56638 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit  
15 to detect 56638 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a  
20 detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein stabilizing agent. The kit can also  
25 include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed  
30 using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of

developing, a disease or disorder associated with misexpressed or aberrant or unwanted 56638 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 56638  
5 expression or activity is identified. A test sample is obtained from a subject and 56638 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 56638 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 56638 expression or activity. As used herein, a "test sample" refers to a biological sample obtained  
10 from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 56638 expression or activity. For example, such methods can be  
15 used to determine whether a subject can be effectively treated with an agent for a disorder as described herein.

In another aspect, the invention features a computer medium having a plurality of digitally encoded data records. Each data record includes a value representing the level of expression of 56638 in a sample, and a descriptor of the sample. The descriptor of the  
20 sample can be an identifier of the sample, a subject from which the sample was derived (e.g., a patient), a diagnosis, or a treatment (e.g., a preferred treatment). In a preferred embodiment, the data record further includes values representing the level of expression of genes other than 56638 (e.g., other genes associated with a 56638-disorder, or other genes on an array). The data record can be structured as a table, e.g., a table that is part of a database  
25 such as a relational database (e.g., a SQL database of the Oracle or Sybase database environments).

Also featured is a method of evaluating a sample. The method includes providing a sample, e.g., from the subject, and determining a gene expression profile of the sample, wherein the profile includes a value representing the level of 56638 expression. The method  
30 can further include comparing the value or the profile (i.e., multiple values) to a reference value or reference profile. The gene expression profile of the sample can be obtained by any



of the methods described herein (e.g., by providing a nucleic acid from the sample and contacting the nucleic acid to an array). The method can be used to diagnose a DISORDERA disorder in a subject wherein an increase in 56638 expression is an indication that the subject has or is disposed to having a disorder as described herein. The method can  
5 be used to monitor a treatment for the disorder in a subject. For example, the gene expression profile can be determined for a sample from a subject undergoing treatment. The profile can be compared to a reference profile or to a profile obtained from the subject prior to treatment or prior to onset of the disorder (see, e.g., Golub *et al.* (1999) *Science* 286:531).

In yet another aspect, the invention features a method of evaluating a test compound  
10 (see also, "Screening Assays", above). The method includes providing a cell and a test compound; contacting the test compound to the cell; obtaining a subject expression profile for the contacted cell; and comparing the subject expression profile to one or more reference profiles. The profiles include a value representing the level of 56638 expression. In a preferred embodiment, the subject expression profile is compared to a target profile, e.g., a  
15 profile for a normal cell or for desired condition of a cell. The test compound is evaluated favorably if the subject expression profile is more similar to the target profile than an expression profile obtained from an uncontacted cell.

In another aspect, the invention features, a method of evaluating a subject. The method includes: a) obtaining a sample from a subject, e.g., from a caregiver, e.g., a  
20 caregiver who obtains the sample from the subject; b) determining a subject expression profile for the sample. Optionally, the method further includes either or both of steps: c) comparing the subject expression profile to one or more reference expression profiles; and d) selecting the reference profile most similar to the subject reference profile. The subject expression profile and the reference profiles include a value representing the level of 56638  
25 expression. A variety of routine statistical measures can be used to compare two reference profiles. One possible metric is the length of the distance vector that is the difference between the two profiles. Each of the subject and reference profile is represented as a multi-dimensional vector, wherein each dimension is a value in the profile.

The method can further include transmitting a result to a caregiver. The result can be  
30 the subject expression profile, a result of a comparison of the subject expression profile with another profile, a most similar reference profile, or a descriptor of any of the aforementioned.

The result can be transmitted across a computer network, e.g., the result can be in the form of a computer transmission, e.g., a computer data signal embedded in a carrier wave.

Also featured is a computer medium having executable code for effecting the following steps: receive a subject expression profile; access a database of reference  
5 expression profiles; and either i) select a matching reference profile most similar to the subject expression profile or ii) determine at least one comparison score for the similarity of the subject expression profile to at least one reference profile. The subject expression profile, and the reference expression profiles each include a value representing the level of 56638 expression.

10

#### Arrays and Uses Thereof

In another aspect, the invention features an array that includes a substrate having a plurality of addresses. At least one address of the plurality includes a capture probe that binds specifically to a 56638 molecule (e.g., a 56638 nucleic acid or a 56638 polypeptide).  
15 The array can have a density of at least 10, 50, 100, 200, 500, 1,000, 2,000, or 10,000 or more addresses/cm<sup>2</sup>, and ranges between. In a preferred embodiment, the plurality of addresses includes at least 10, 100, 500, 1,000, 5,000, 10,000, 50,000 addresses. In a preferred embodiment, the plurality of addresses includes equal to or less than 10, 100, 500, 1,000, 5,000, 10,000, or 50,000 addresses. The substrate can be a two-dimensional substrate  
20 such as a glass slide, a wafer (e.g., silica or plastic), a mass spectroscopy plate, or a three-dimensional substrate such as a gel pad. Addresses in addition to address of the plurality can be disposed on the array.

In a preferred embodiment, at least one address of the plurality includes a nucleic acid capture probe that hybridizes specifically to a 56638 nucleic acid, e.g., the sense or anti-sense  
25 strand. In one preferred embodiment, a subset of addresses of the plurality of addresses has a nucleic acid capture probe for 56638. Each address of the subset can include a capture probe that hybridizes to a different region of a 56638 nucleic acid. In another preferred embodiment, addresses of the subset include a capture probe for a 56638 nucleic acid. Each address of the subset is unique, overlapping, and complementary to a different variant of  
30 56638 (e.g., an allelic variant, or all possible hypothetical variants). The array can be used to

sequence 56638 by hybridization (see, e.g., U.S. Patent No. 5,695,940).

An array can be generated by various methods, e.g., by photolithographic methods (see, e.g., U.S. Patent Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g., directed-flow methods as described in U.S. Patent No. 5,384,261), pin-based methods (e.g.,  
5 as described in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

In another preferred embodiment, at least one address of the plurality includes a polypeptide capture probe that binds specifically to a 56638 polypeptide or fragment thereof. The polypeptide can be a naturally-occurring interaction partner of 56638 polypeptide.

10 Preferably, the polypeptide is an antibody, e.g., an antibody described herein (see "Anti-56638 Antibodies," above), such as a monoclonal antibody or a single-chain antibody.

In another aspect, the invention features a method of analyzing the expression of 56638. The method includes providing an array as described above; contacting the array with a sample and detecting binding of a 56638-molecule (e.g., nucleic acid or polypeptide)  
15 to the array. In a preferred embodiment, the array is a nucleic acid array. Optionally the method further includes amplifying nucleic acid from the sample prior or during contact with the array.

In another embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array, particularly the expression of 56638. If a  
20 sufficient number of diverse samples is analyzed, clustering (e.g., hierarchical clustering, k-means clustering, Bayesian clustering and the like) can be used to identify other genes which are co-regulated with 56638. For example, the array can be used for the quantitation of the expression of multiple genes. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertained. Quantitative data can be used to  
25 group (e.g., cluster) genes on the basis of their tissue expression *per se* and level of expression in that tissue.

For example, array analysis of gene expression can be used to assess the effect of cell-cell interactions on 56638 expression. A first tissue can be perturbed and nucleic acid from a second tissue that interacts with the first tissue can be analyzed. In this context, the  
30 effect of one cell type on another cell type in response to a biological stimulus can be determined, e.g., to monitor the effect of cell-cell interaction at the level of gene expression.

In another embodiment, cells are contacted with a therapeutic agent. The expression profile of the cells is determined using the array, and the expression profile is compared to the profile of like cells not contacted with the agent. For example, the assay can be used to determine or analyze the molecular basis of an undesirable effect of the therapeutic agent. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor expression of one or more genes in the array with respect to time. For example, samples obtained from different time points can be probed with the array. Such analysis can identify and/or characterize the development of a 56638-associated disease or disorder; and processes, such as a cellular transformation associated with a 56638-associated disease or disorder. The method can also evaluate the treatment and/or progression of a 56638-associated disease or disorder

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including 56638) that could serve as a molecular target for diagnosis or therapeutic intervention.

In another aspect, the invention features an array having a plurality of addresses. Each address of the plurality includes a unique polypeptide. At least one address of the plurality has disposed thereon a 56638 polypeptide or fragment thereof. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt *et al.* (2000). *Nature Biotech.* 18, 989-994; Lueking *et al.* (1999). *Anal. Biochem.* 270, 103-111; Ge, H. (2000). *Nucleic Acids Res.* 28, e3, I-VII; MacBeath, G., and Schreiber, S.L. (2000). *Science* 289, 1760-1763; and WO 99/51773A1. In a preferred embodiment, each addresses of the plurality has disposed thereon a polypeptide at least 60, 70, 80, 85, 90, 95 or 99 % identical to a 56638 polypeptide or fragment thereof. For example, multiple variants of a 56638 polypeptide (e.g., encoded by allelic variants, site-directed mutants, random mutants, or combinatorial mutants) can be disposed at individual addresses of the plurality. Addresses in addition to the address of the plurality can be disposed on the array.

The polypeptide array can be used to detect a 56638 binding compound, e.g., an antibody in a sample from a subject with specificity for a 56638 polypeptide or the presence of a 56638-binding protein or ligand.

5 The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (e.g., ascertaining the effect of 56638 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

In another aspect, the invention features a method of analyzing a plurality of probes.  
10 The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express 56638 or from a cell or subject in which a 56638 mediated response has been elicited, e.g., by contact  
15 of the cell with 56638 nucleic acid or protein, or administration to the cell or subject 56638 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express 56638 (or does not express  
20 as highly as in the case of the 56638 positive plurality of capture probes) or from a cell or subject which in which a 56638 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a 56638 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid,  
25 hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features a method of analyzing a plurality of probes or a sample. The method is useful, e.g., for analyzing gene expression. The method  
includes: providing a two dimensional array having a plurality of addresses, each address of  
30 the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, contacting the array with a first sample from a cell or subject

which express or mis-express 56638 or from a cell or subject in which a 56638-mediated response has been elicited, e.g., by contact of the cell with 56638 nucleic acid or protein, or administration to the cell or subject 56638 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being  
5 positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, and contacting the array with a second sample from a cell or subject which does not express 56638 (or does not express as highly as in the case of the 56638 positive plurality of capture probes) or from a cell or subject in which a 56638 mediated response has not been elicited (or has been elicited to a lesser extent than in  
10 the first sample); and comparing the binding of the first sample with the binding of the second sample. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody. The same array can be used for both samples or different arrays can be used. If different arrays are used the plurality of addresses  
15 with capture probes should be present on both arrays.

In another aspect, the invention features a method of analyzing 56638, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a 56638 nucleic acid or amino acid sequence; comparing the 56638 sequence with one or more preferably a plurality of sequences from a collection of  
20 sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze 56638.

#### Detection of Variations or Mutations

The methods of the invention can also be used to detect genetic alterations in a 56638 gene, thereby determining if a subject with the altered gene is at risk for a disorder  
25 characterized by misregulation in 56638 protein activity or nucleic acid expression, such as a disorder as described herein. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 56638-protein, or the mis-expression of the 56638 gene. For example, such genetic alterations can be detected by  
30 ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 56638 gene; 2) an addition of one or more nucleotides to a 56638 gene; 3) a substitution of

one or more nucleotides of a 56638 gene, 4) a chromosomal rearrangement of a 56638 gene; 5) an alteration in the level of a messenger RNA transcript of a 56638 gene, 6) aberrant modification of a 56638 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 56638 gene, 8) a non-wild type level of a 56638-protein, 9) allelic loss of a 56638 gene, and 10) inappropriate post-translational modification of a 56638-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the 56638-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 56638 gene under conditions such that hybridization and amplification of the 56638-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein. Alternatively, other amplification methods described herein or known in the art can be used.

In another embodiment, mutations in a 56638 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 56638 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, two-dimensional arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. A probe can be complementary to a region of a 56638 nucleic acid or a putative variant (e.g.,

allelic variant) thereof. A probe can have one or more mismatches to a region of a 56638 nucleic acid (e.g., a destabilizing mismatch). The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 56638 can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 56638 gene and detect mutations by comparing the sequence of the sample 56638 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

Other methods for detecting mutations in the 56638 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242; Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295).

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 56638 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 56638 genes. For example, single strand conformation polymorphism (SSCP)



may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 56638 nucleic acids will be denatured and  
5 allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a  
10 preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient  
15 gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and  
20 Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). A further method of detecting point mutations is the chemical ligation of  
25 oligonucleotides as described in Xu *et al.* ((2001) *Nature Biotechnol.* 19:148). Adjacent oligonucleotides, one of which selectively anneals to the query site, are ligated together if the nucleotide at the query site of the sample nucleic acid is complementary to the query oligonucleotide; ligation can be monitored, e.g., by fluorescent dyes coupled to the oligonucleotides.

30 Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used

as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

In another aspect, the invention features a set of oligonucleotides. The set includes a plurality of oligonucleotides, each of which is at least partially complementary (e.g., at least 50%, 60%, 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 99% complementary) to a 56638 nucleic acid.

In a preferred embodiment the set includes a first and a second oligonucleotide. The first and second oligonucleotide can hybridize to the same or to different locations of SEQ ID NO:1 or 3 or the complement of SEQ ID NO:1 or 3. Different locations can be different but overlapping or nonoverlapping on the same strand. The first and second oligonucleotide can hybridize to sites on the same or on different strands.

The set can be useful, e.g., for identifying SNP's, or identifying specific alleles of 56638. In a preferred embodiment, each oligonucleotide of the set has a different nucleotide at an interrogation position. In one embodiment, the set includes two oligonucleotides, each complementary to a different allele at a locus, e.g., a biallelic or polymorphic locus.

In another embodiment, the set includes four oligonucleotides, each having a different nucleotide (e.g., adenine, guanine, cytosine, or thymidine) at the interrogation position. The interrogation position can be a SNP or the site of a mutation. In another preferred embodiment, the oligonucleotides of the plurality are identical in sequence to one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotide that hybridizes to one allele provides a signal that is distinguishable from an oligonucleotide that hybridizes to a second allele. In still another

embodiment, at least one of the oligonucleotides of the set has a nucleotide change at a position in addition to a query position, e.g., a destabilizing mutation to decrease the  $T_m$  of the oligonucleotide. In another embodiment, at least one oligonucleotide of the set has a non-natural nucleotide, e.g., inosine. In a preferred embodiment, the oligonucleotides are  
5 attached to a solid support, e.g., to different addresses of an array or to different beads or nanoparticles.

In a preferred embodiment the set of oligo nucleotides can be used to specifically amplify, e.g., by PCR, or detect, a 56638 nucleic acid.

The methods described herein may be performed, for example, by utilizing pre-  
10 packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 56638 gene.

#### Use of 56638 Molecules as Surrogate Markers

The 56638 molecules of the invention are also useful as markers of disorders or  
15 disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 56638 molecules of the invention may be detected, and may be correlated with one or more biological states in vivo. For example, the 56638 molecules of the invention may serve as  
20 surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers  
25 may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be  
30 made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be

made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

5           The 56638 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of  
10   the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic  
15   marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a  
20   56638 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-56638 antibodies may be employed in an immune-based detection system for a 56638 protein marker, or 56638-specific radiolabeled probes may be used to detect a 56638 mRNA marker.  
25   Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.  
30           The 56638 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a “pharmacogenomic marker” is an objective biochemical marker which

correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35:1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 56638 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 56638 DNA may correlate 56638 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

#### Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-56638 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as

ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the

active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of

such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.



As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al. ((1997) *J Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is  
5 furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at  
10 first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or  
15 activity to be modulated.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine,  
20 colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, e.g., maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545) and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g.,  
25 methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g.,  
30 dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids). Radioactive

ions include, but are not limited to iodine, yttrium and praseodymium.

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) *Proc Natl Acad Sci USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

#### Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted expression or activity. "Treatment" or "treating a subject" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to

cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

5           With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term  
10           refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype," or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 56638 molecules of the present invention or 56638 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician  
15           or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

          In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 56638 expression or activity, by  
20           administering to the subject a 56638 or an agent which modulates 56638 expression or at least one 56638 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 56638 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the  
25           56638 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 56638 aberrance, for example, a 56638 agonist or 56638 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

          As the 56638 mRNA is expressed in human adrenal gland, brain, heart, kidney, liver,  
30           lung, mammary gland, placenta, prostate, salivary gland, muscle, small intestine, spleen, stomach, testes, thymus, trachea, uterus, spinal cord, skin, and dorsal root ganglion (DRG),

the molecules of the invention can be used to treat, prevent, and/or diagnose disorders involving activities of those cells. In addition to the disorders described above, the molecules of the invention can be effective therapeutic/diagnostic agents of cardiovascular, renal, digestive, muscular, intestinal, among other disorders.

5           Examples of disorders involving the heart or "cardiovascular disorder" include, but are not limited to, a disease, disorder, or state involving the cardiovascular system, *e.g.*, the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel, *e.g.*, by a thrombus. Examples of such disorders include hypertension, atherosclerosis,  
10       coronary artery spasm, congestive heart failure, coronary artery disease, valvular disease, arrhythmias, and cardiomyopathies.

          It is possible that some 56638 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the  
15       amelioration of disorder symptoms. For example, in pain, pain related disorders, inflammatory disorders, disorders of the trachea, the male reproductive system, or the hemotopoietic system.

          As discussed, successful treatment of 56638 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For  
20       example, compounds, *e.g.*, an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 56638 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or  
25       single chain antibodies, and Fab, F(ab')<sub>2</sub> and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof).

          Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix  
30       molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 56638 expression is through the use of aptamer molecules specific for 56638 protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne et al. (1997) *Curr Opin Chem Biol* 1(1): 5-9; and Patel (1997) *Curr Opin Chem Biol* 1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which 56638 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in instances whereby negative modulatory techniques are appropriate for the treatment of 56638 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a 56638 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 56638 through the use of anti-idiotypic antibodies (see, for example, Herlyn (1999) *Ann Med* 31(1):66-78; and Bhattacharya-Chatterjee and Foon (1998) *Cancer Treat Res* 94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 56638 protein. Vaccines directed to a disease characterized by 56638 expression may also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used,

internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (see e.g., Marasco et al. (1993) *Proc Natl Acad Sci USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or ameliorate disorders, e.g., to control pain, pain related disorders, inflammatory disorders, disorders of the trachea, the male reproductive system, or the hemotopoietic system. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma

concentration range that includes the IC<sub>50</sub> (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

5           Another example of determination of effective dose for an individual is the ability to directly assay levels of “free” and “bound” compound in the serum of the test subject. Such assays may utilize antibody mimics and/or “biosensors” that have been created through molecular imprinting techniques. The compound which is able to modulate 56638 activity is used as a template, or “imprinting molecule”, to spatially organize polymerizable monomers  
10 prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell et al (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea (1994) *Trends in Polymer Science* 2:166-173.  
15 Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis et al (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the “free” concentration of compound which modulates the expression or activity of 56638 can be readily monitored and  
20 used in calculations of IC<sub>50</sub>.

Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate  
25 fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC<sub>50</sub>. An rudimentary example of such a “biosensor” is discussed in Kriz et al. (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 56638 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 56638 or agent that  
30 modulates one or more of the activities of 56638 protein activity associated with the cell. An agent that modulates 56638 protein activity can be an agent as described herein, such as a



nucleic acid or a protein, a naturally-occurring target molecule of a 56638 protein (e.g., a 56638 substrate or receptor), a 56638 antibody, a 56638 agonist or antagonist, a peptidomimetic of a 56638 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or 56638 activities. Examples of such stimulatory agents include active 56638 protein and a nucleic acid molecule encoding 56638. In another embodiment, the agent inhibits one or more 56638 activities. Examples of such inhibitory agents include antisense 56638 nucleic acid molecules, anti 56638 antibodies, and 56638 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 56638 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up regulates or down regulates) 56638 expression or activity. In another embodiment, the method involves administering a 56638 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 56638 expression or activity.

Stimulation of 56638 activity is desirable in situations in which 56638 is abnormally downregulated and/or in which increased 56638 activity is likely to have a beneficial effect. For example, stimulation of 56638 activity is desirable in situations in which a 56638 is downregulated and/or in which increased 56638 activity is likely to have a beneficial effect. Likewise, inhibition of 56638 activity is desirable in situations in which 56638 is abnormally upregulated and/or in which decreased 56638 activity is likely to have a beneficial effect.

#### Pharmacogenomics

The 56638 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 56638 activity (e.g., 56638 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 56638 associated disorders (e.g., pain, pain related disorders, inflammatory disorders, disorders of the trachea, the male reproductive system, or the hemotopoietic system) associated with aberrant or unwanted 56638 activity. In

conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 56638 molecule or 56638 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 56638 molecule or 56638 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum et al. (1996) *Clin Exp Pharmacol Physiol* 23(10-11) :983-985 and Linder et al. (1997) *Clin Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every

1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a 56638 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 56638 molecule or 56638 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 56638 molecule or 56638 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 56638 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 56638 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., human cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a

56638 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 56638 gene expression, protein levels, or upregulate 56638 activity, can be monitored in clinical trials of subjects exhibiting decreased 56638 gene expression, protein levels, or downregulated 56638 activity.

5 Alternatively, the effectiveness of an agent determined by a screening assay to decrease 56638 gene expression, protein levels, or downregulate 56638 activity, can be monitored in clinical trials of subjects exhibiting increased 56638 gene expression, protein levels, or upregulated 56638 activity. In such clinical trials, the expression or activity of a 56638 gene, and preferably, other genes that have been implicated in, for example, a 56638-associated  
10 disorder can be used as a "read out" or markers of the phenotype of a particular cell.

#### 56638 Informatics

The sequence of a 56638 molecule is provided in a variety of media to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or amino acid molecule, which contains a 56638. Such a manufacture can provide a nucleotide  
15 or amino acid sequence, e.g., an open reading frame, in a form which allows examination of the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form. The sequence information can include, but is not limited to, 56638 full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including  
20 single nucleotide polymorphisms (SNPs), epitope sequence, and the like. In a preferred embodiment, the manufacture is a machine-readable medium, e.g., a magnetic, optical, chemical or mechanical information storage device.

As used herein, "machine-readable media" refers to any medium that can be read and accessed directly by a machine, e.g., a digital computer or analogue computer. Non-limiting  
25 examples of a computer include a desktop PC, laptop, mainframe, server (e.g., a web server, network server, or server farm), handheld digital assistant, pager, mobile telephone, and the like. The computer can be stand-alone or connected to a communications network, e.g., a local area network (such as a VPN or intranet), a wide area network (e.g., an Extranet or the Internet), or a telephone network (e.g., a wireless, DSL, or ISDN network). Machine-  
30 readable media include, but are not limited to: magnetic storage media, such as floppy discs,

hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM, ROM, EPROM, EEPROM, flash memory, and the like; and hybrids of these categories such as magnetic/optical storage media.

5 A variety of data storage structures are available to a skilled artisan for creating a machine-readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a  
10 word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence  
15 information of the present invention.

In a preferred embodiment, the sequence information is stored in a relational database (such as Sybase or Oracle). The database can have a first table for storing sequence (nucleic acid and/or amino acid sequence) information. The sequence information can be stored in one field (*e.g.*, a first column) of a table row and an identifier for the sequence can be store in  
20 another field (*e.g.*, a second column) of the table row. The database can have a second table, *e.g.*, storing annotations. The second table can have a field for the sequence identifier, a field for a descriptor or annotation text (*e.g.*, the descriptor can refer to a functionality of the sequence, a field for the initial position in the sequence to which the annotation refers, and a field for the ultimate position in the sequence to which the annotation refers. Non-limiting  
25 examples for annotation to nucleic acid sequences include polymorphisms (*e.g.*, SNP's) translational regulatory sites and splice junctions. Non-limiting examples for annotations to amino acid sequence include polypeptide domains, *e.g.*, a domain described herein; active sites and other functional amino acids; and modification sites.

By providing the nucleotide or amino acid sequences of the invention in computer  
30 readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid

sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif. The search can be a BLAST search or other  
5 routine sequence comparison, e.g., a search described herein.

Thus, in one aspect, the invention features a method of analyzing 56638, e.g., analyzing structure, function, or relatedness to one or more other nucleic acid or amino acid sequences. The method includes: providing a 56638 nucleic acid or amino acid sequence; comparing the 56638 sequence with a second sequence, e.g., one or more preferably a  
10 plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database to thereby analyze 56638. The method can be performed in a machine, e.g., a computer, or manually by a skilled artisan.

The method can include evaluating the sequence identity between a 56638 sequence and a database sequence. The method can be performed by accessing the database at a  
15 second site, e.g., over the Internet.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10  
20 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium for analysis and comparison  
25 to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBI).

Thus, the invention features a method of making a computer readable record of a  
30 sequence of a 56638 sequence which includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the following:

identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention features, a method of analyzing a sequence. The method includes: providing a 56638 sequence, or record, in machine-readable form; comparing a second sequence to the 56638 sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, e.g., determining if the 56638 sequence includes a sequence being compared. In a preferred embodiment the 56638 or second sequence is stored on a first computer, e.g., at a first site and the comparison is performed, read, or recorded on a second computer, e.g., at a second site. E.g., the 56638 or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention provides a machine-readable medium for holding instructions for performing a method for determining whether a subject has a 56638-associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder, wherein the method comprises the steps of determining 56638 sequence information associated with the subject and based on the 56638 sequence information, determining whether the subject has a 56638-associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 56638-associated disease or disorder or a pre-disposition to a disease associated with a 56638 wherein the method comprises the steps of determining 56638 sequence information associated with the subject, and based on the 56638 sequence information, determining whether the subject has a 56638-associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder, and/or recommending

a particular treatment for the disease, disorder or pre-disease condition. In a preferred embodiment, the method further includes the step of receiving information, e.g., phenotypic or genotypic information, associated with the subject and/or acquiring from a network phenotypic information associated with the subject. The information can be stored in a database, e.g., a relational database. In another embodiment, the method further includes accessing the database, e.g., for records relating to other subjects, comparing the 56638 sequence of the subject to the 56638 sequences in the database to thereby determine whether the subject as a 56638-associated disease or disorder, or a pre-disposition for such.

The present invention also provides in a network, a method for determining whether a subject has a 56638 associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder associated with 56638, said method comprising the steps of receiving 56638 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 56638 and/or corresponding to a 56638-associated disease or disorder (e.g., pain related disorders, inflammatory disorders, disorders of the trachea, the male reproductive system, or the hematopoietic system), and based on one or more of the phenotypic information, the 56638 information (e.g., sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 56638-associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a method for determining whether a subject has a 56638 -associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder, said method comprising the steps of receiving information related to 56638 (e.g., sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 56638 and/or related to a 56638-associated disease or disorder, and based on one or more of the phenotypic information, the 56638 information, and the acquired information, determining whether the subject has a 56638-associated disease or disorder or a pre-disposition to a 56638-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.



This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

## EXAMPLES

### 5 Example 1: Identification and Characterization of Human 56638 cDNA

The human 56638 sequence (Fig. 1; SEQ ID NO:1), which is approximately 2953 nucleotides long, including untranslated regions, contains a predicted methionine-initiated coding sequence of about 2340 nucleotides, including the termination codon (nucleotides indicated as "coding" of SEQ ID NO:1 in Fig. 1; SEQ ID NO:3). The coding sequence  
10 encodes a 779 amino acid protein (SEQ ID NO:2).

### Example 2: Tissue Distribution of 56638 mRNA

Endogenous human 56638 gene expression was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System which employs TaqMan technology. Briefly, TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific  
15 oligonucleotide (referred to as a probe) which has a fluorescent dye coupled to its 5' end (typically 6-FAM) and a quenching dye at the 3' end (typically TAMRA). When the fluorescently tagged oligonucleotide is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of Taq polymerase digests the labeled primer, producing a free nucleotide labeled with 6-FAM, which is now detected as a  
20 fluorescent signal. The PCR cycle where fluorescence is first released and detected is directly proportional to the starting amount of the gene of interest in the test sample, thus providing a way of quantitating the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe specific for a housekeeping gene such as GAPDH which has been labeled with a different fluorophore on the 5' end (typically  
25 VIC).

To determine the level of 56638 in various human tissues a primer/probe set was designed using Primer Express (Perkin-Elmer) software and primary cDNA sequence information. Total RNA was prepared from a series of human tissues using an RNeasy kit

from Qiagen. First strand cDNA was prepared from 1 µg total RNA using an oligo-dT primer and Superscript II reverse transcriptase (Gibco/BRL). cDNA obtained from approximately 50 ng total RNA was used per TaqMan reaction. 56638 mRNA levels were analyzed in a variety of samples of human tissues, and in rodent models of pain response.

5           Figure 4 shows relative 56638 mRNA expression on mRNA derived from human tissue samples, both normal, and tumor. The samples are derived from human adrenal gland, brain, heart, kidney, liver, lung, mammary gland, placenta, prostate, salivary gland, muscle, small intestine, spleen, stomach, testes, thymus, trachea, uterus, spinal cord, skin, and dorsal root ganglion (DRG). The highest 56638 mRNA expression was observed in spinal cord,  
10       DRG, small intestine, testes, and trachea.

Taqman experiments in rat showed that 56638 is expressed in pituitary gland, spinal cord, brain, nerve, TRG and DRG.

Taqman experiments on rodent models of pain response showed that the 56638 gene is up-regulated at least 4-fold in DRG 7 days after axotomy (AXT) and at least 5-fold in the  
15       chronic constriction injury (CCI) model of neuropathic pain (7 days). (See Table 1 below). No regulation was observed in the model of inflammatory pain (CFA), and there was no regulation in rat spinal cord in any of the models analyzed.

**Table 1: Time Course Of 56638 Expression In Pain Response**

20	<u>Tissue</u>	<u>Expression</u>
	Naïve DRG (neg.control)	0.004
	I DRG CCI 3 days	0.004
	I DRG CCI 7 days	0.022
	I DRG CCI 14 days	0.004
25	I DRG CCI 10 days	0.004
	I DRG CCI 28 days	0.002
	I DRG CFA 1 day	0.002
	I DRG CFA 3 days	0.003
	I DRG CFA 7 days	0.002
30	I DRG CFA 14 days	0.001
	I DRG CFA 28 days	0.003
	I DRG AXT 1 day	0.003
	I DRG AXT 3 days	0.002
	I DRG AXT 7 days	0.017
35	I DRG AXT 14 days	0.004
	I DRG AXT 28 days	0.003

*In situ* hybridization experiments with the human 56638 probe showed expression in monkey brain, a subpopulation of DRG neurons, in the epithelium of trachea, and small intestine, as well as skin. *In situ* hybridization in rat animal models shows up-regulation of the 56638 gene one and seven days after axotomy and after CFA intraplantar injection.

5 These levels go back to normal at later time points. No contralateral effects were observed.

In addition, the relative expression level of expression of 56638 in various normal versus cancer tissues was determined by Taqman analysis (see Table 2 below). The highest amount of expression was found in placenta.

10 **Table 2: Oncology Panel:**

	<u>Tissue Type</u>	<u>Relative Expression</u>
	CHT 523 Colon N	0.00
	NDR 104 Colon N	0.00
	CHT 416 Colon N	0.94
15	CHT 452 Colon N	0.08
	NDR 210 Colon T	0.57
	CHT 398 Colon T	1.26
	CHT 382 Colon T	0.00
	CHT 944 Colon T	0.00
20	CHT 528 Colon T	1.99
	CHT 368 Colon T	0.11
	CHT 372 Colon T	0.37
	CLN 609 Colon T	0.03
	CHT 01 Liver Met	0.02
25	CHT 3 Liver Met	0.23
	CHT 340 Liver Met	0.00
	NDR 217 Liver Met	0.34
	PIT 260 Liver N	0.00
	CHT 320 Liver N	0.00
30	A4 HMVEC-Arr	0.04
	C48 HMVEC-Prol	1.04
	CHT 50 Placenta	6.17
	ONC 102 Hemangioma	0.00

35 Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 56638 cDNA (SEQ ID NO:1) can be used. The DNA can be radioactively labeled with <sup>32</sup>P-dCTP using the Prime-It Kit (Stratagene, La

Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

5     Example 3: Recombinant Expression of 56638 in Bacterial Cells

In this example, 56638 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 56638 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-56638 fusion protein in PEB199 is induced with  
10     IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

Example 4: Expression of Recombinant 56638 Protein in COS Cells

15     To express the 56638 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 56638 protein and an HA tag (Wilson et al. (1984) *Cell* 37:767) or a FLAG tag  
20     fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 56638 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately  
25     twenty nucleotides of the 56638 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 56638 coding sequence. The PCR amplified fragment and the pcDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP

enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the 56638 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 $\alpha$ , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 56638-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the 56638 polypeptide is detected by radiolabelling (35S-methionine or 35S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with 35S-methionine (or 35S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 56638 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 56638 polypeptide is detected by radiolabelling and immunoprecipitation using a 56638 specific monoclonal antibody.

### Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:
  - a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1 or 3; and
  - b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2.
2. The nucleic acid molecule of claim 1, further comprising vector nucleic acid sequences.
3. The nucleic acid molecule of claim 1, further comprising nucleic acid sequences encoding a heterologous polypeptide.
4. A host cell that contains the nucleic acid molecule of claim 1.
5. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.
6. The polypeptide of claim 5, further comprising heterologous amino acid sequences.
7. An antibody, or antigen-binding fragment thereof, that selectively binds to a polypeptide of claim 5.
8. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:2, the method comprising culturing the host cell of claim 4 under conditions in which the nucleic acid molecule is expressed.
9. A method for detecting the presence of a polypeptide of claim 5 in a sample, comprising:
  - a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and
  - b) determining whether the compound binds to the polypeptide in the sample.

10. The method of claim 9, wherein the compound that binds to the polypeptide is an antibody.
11. A kit comprising a compound which selectively binds to a polypeptide of claim 5 and instructions for use.
12. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising the steps of:
  - a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
  - b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample.
13. The method of claim 12, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.
14. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.
15. A method for identifying a compound which binds to a polypeptide of claim 5 comprising the steps of:
  - a) contacting a polypeptide, or a cell expressing a polypeptide of claim 5 with a test compound; and
  - b) determining whether the polypeptide binds to the test compound.
16. A method for modulating the activity of a polypeptide of claim 5, comprising contacting a polypeptide or a cell expressing a polypeptide of claim 5 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.
17. A method of inhibiting aberrant activity of a 58824-expressing cell, comprising

contacting a 58824-expressing cell with a compound that modulates the activity or expression of a polypeptide of claim 5, in an amount which is effective to reduce or inhibit the aberrant activity of the cell.

18. The method of claim 17, wherein the compound is selected from the group consisting of a peptide, a phosphopeptide, a small organic molecule, and an antibody.

19. The method of claim 17, wherein the cell is located in an area involved in pain control.

20. A method of modulating a pain response in a subject, comprising:  
administering to the subject an effective amount of a compound that modulates the activity or expression of a polypeptide of claim 5, such that the pain response in the subject is modulated.



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START SEQ ID NO:1

cggacgcgtg ggcggaagcc ttcaggcagc ctcaactcag ccccaagcca ctgctctccc 6  
 atcccagttc ctggaaatcc acccacttgg cccagctcac cccaactcca acccactggg 12  
 acccagtctc caggggcctg actgtgggcg gcagccactc ctgagtgagc aaagggttcct 18  
 ccgcgggtgct ctcccgtcca gagccctgct g atg ggg aag tcc gaa ggc ccc 23  
 Met Gly Lys Ser Glu Gly Pro 5

START SEQ ID NO:3

START SEQ ID NO:2

gtg ggg atg gtg gag agc gct ggc cgt gca ggg cag aag cgc ccg ggg 28  
 Val Gly Met Val Glu Ser Ala Gly Arg Ala Gly Gln Lys Arg Pro Gly 10 15 20

ttc ctg gag ggg ggg ctg ctg ctg ctg ctg ctg gtg acc gct gcc 32  
 Phe Leu Glu Gly Gly Leu Leu Leu Leu Leu Leu Val Thr Ala Ala 25 30 35

ctg gtg gcc ttg ggt gtc ctc tac gcc gac cgc aga ggg aag cag ctg 37  
 Leu Val Ala Leu Gly Val Leu Tyr Ala Asp Arg Arg Gly Lys Gln Leu 40 45 50 55

cca cgc ctt gct agc cgg ctg tgc ttc tta cag gag gag agg acc ttt 42  
 Pro Arg Leu Ala Ser Arg Leu Cys Phe Leu Gln Glu Glu Arg Thr Phe 60 65 70

gta aaa cga aaa ccc cga ggg atc cca gag gcc caa gag gtg agc gag 47  
 Val Lys Arg Lys Pro Arg Gly Ile Pro Glu Ala Gln Glu Val Ser Glu 75 80 85

gtc tgc acc acc cct ggc tgc gtg ata gca gct gcc agg atc ctc cag 52  
 Val Cys Thr Thr Pro Gly Cys Val Ile Ala Ala Ala Arg Ile Leu Gln 90 95 100

aac atg gac ccg acc acg gaa ccg tgt gac gac ttc tac cag ttt gca 56  
 Asn Met Asp Pro Thr Thr Glu Pro Cys Asp Asp Phe Tyr Gln Phe Ala 105 110 115

tgc gga ggc tgg ctg cgg cgc cac gtg atc cct gag acc aac tca aga 61  
 Cys Gly Gly Trp Leu Arg Arg His Val Ile Pro Glu Thr Asn Ser Arg 120 125 130 135

tac agc atc ttt gac gtc ctc cgc gac gag ctg gag gtc atc ctc aaa 66  
 Tyr Ser Ile Phe Asp Val Leu Arg Asp Glu Leu Glu Val Ile Leu Lys 140 145 150

gcg gtg ctg gag aat tcg act gcc aag gac cgg ccg gct gtg gag aag 71  
 Ala Val Leu Glu Asn Ser Thr Ala Lys Asp Arg Pro Ala Val Glu Lys 155 160 165

gcc agg acg ctg tac cgc tcc tgc atg aac cag agt gtg ata gag aag 76  
 Ala Arg Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys 170 175 180

cga ggc tct cag ccc ctg ctg gac att ttg gag gtg gtg gga ggc tgg 81  
 Arg Gly Ser Gln Pro Leu Leu Asp Ile Leu Glu Val Val Gly Gly Trp 185 190 195

ccg gtg gcg atg gac agg tgg aac gag acc gta gga ctc gag tgg gag 86  
 Pro Val Ala Met Asp Arg Trp Asn Glu Thr Val Gly Leu Glu Trp Glu 200- 205 210 215

Fin 1A

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ctg gag cgg cag ctg gcg ctg atg aac tca cag ttc aac agg cgc gtc Leu Glu Arg Gln Leu Ala Leu Met Asn Ser Gln Phe Asn Arg Arg Val 220 225 230	904
ctc atc gac ctc ttc atc tgg aac gac gac cag aac tcc agc cgg cac Leu Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His 235 240 245	952
atc atc tac ata gac cag ccc acc ttg ggc atg ccc tcc cga gag tac Ile Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr 250 255 260	1000
tac ttc aac ggc ggc agc aac cgg aag gtg cgg gaa gcc tac ctg cag Tyr Phe Asn Gly Gly Ser Asn Arg Lys Val Arg Glu Ala Tyr Leu Gln 265 270 275	1048
ttc atg gtg tca gtg gcc acg ttg ctg cgg gag gat gca aac ctg ccc Phe Met Val Ser Val Ala Thr Leu Leu Arg Glu Asp Ala Asn Leu Pro 280 285 290 295	1096
agg gac agc tgc ctg gtg cag gag gac atg gtg cag gtg ctg gag ctg Arg Asp Ser Cys Leu Val Gln Glu Asp Met Val Gln Val Leu Glu Leu 300 305 310	1144
gag aca cag ctg gcc aag gcc acg gta ccc cag gag gag aga cac gac Glu Thr Gln Leu Ala Lys Ala Thr Val Pro Gln Glu Glu Arg His Asp 315 320 325	1192
gtc atc gcc ttg tac cac cgg atg gga ctg gag gag ctg caa agc cag Val Ile Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Ser Gln 330 335 340	1240
ttt ggc ctg aag gga ttt aac tgg act ctg ttc ata caa act gtg cta Phe Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu 345 350 355	1288
tcc tct gtc aaa atc aag ctg ctg cca gat gag gaa gtg gtg gtc tat Ser Ser Val Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Val Tyr 360 365 370 375	1336
ggc atc ccc tac ctg cag aac ctt gaa aac atc atc gac acc tac tca Gly Ile Pro Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser 380 385 390	1384
gcc agg acc ata cag aac tac ctg gtc tgg cgc ctg gtg ctg gac cgc Ala Arg Thr Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg 395 400 405	1432
att ggt agc cta agc cag aga ttc aag gac aca cga gtg aac tac cgc Ile Gly Ser Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg 410 415 420	1480
aag gcg ctg ttt ggc aca atg gtg gag gag gtg cgc tgg cgt gaa tgt Lys Ala Leu Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys 425 430 435	1528
gtg ggc tac gtc aac agc aac atg gag aac gcc gtg ggc tcc ctc tac Val Gly Tyr Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr 440 445 450 455	1576

Fig. 1B

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gtc agg gag gcg ttc cct gga gac agc aag agc atg gtc aga gaa ctc	162
Val Arg Glu Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu	
460 465 470	
att gac aag gtg cgg aca gtg ttt gtg gag acg ctg gac gag ctg ggc	167
Ile Asp Lys Val Arg Thr Val Phe Val Glu Thr Leu Asp Glu Leu Gly	
475 480 485	
tgg atg gac gag gag tcc aag aag aag gcg cag gag aag gcc atg agc	172
Trp Met Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Met Ser	
490 495 500	
atc cgg gag cag atc ggg cac cct gac tac atc ctg gag gag atg aac	176
Ile Arg Glu Gln Ile Gly His Pro Asp Tyr Ile Leu Glu Glu Met Asn	
505 510 515	
agg cgc ctg gac gag gag tac tcc aat ctg aac ttc tca gag gac ctg	181
Arg Arg Leu Asp Glu Glu Tyr Ser Asn Leu Asn Phe Ser Glu Asp Leu	
520 525 530 535	
tac ttt gag aac agt ctg cag aac ctc aag gtg ggc gcc cag cgg agc	186
Tyr Phe Glu Asn Ser Leu Gln Asn Leu Lys Val Gly Ala Gln Arg Ser	
540 545 550	
ctc agg aag ctt cgg gaa aag gtg gac cca aat ctc tgg atc atc ggg	191
Leu Arg Lys Leu Arg Glu Lys Val Asp Pro Asn Leu Trp Ile Ile Gly	
555 560 565	
gcg gcg gtg gtc aat gcg ttc tac tcc cca aac cga aac cag att gta	196
Ala Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln Ile Val	
570 575 580	
ttc cct gcc ggg atc ctc cag ccc ccc ttc ttc agc aag gag cag cca	200
Phe Pro Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Glu Gln Pro	
585 590 595	
cag gcc ttg aac ttt gga ggc att ggg atg gtg atc ggg cac gag atc	205
Gln Ala Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile	
600 605 610 615	
acg cac ggc ttt gac gac aat ggc cgg aac ttc gac aag aat ggc aac	210
Thr His Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn Gly Asn	
620 625 630	
atg atg gat tgg tgg agt aac ttc tcc acc cag cac ttc cgg gag cag	215
Met Met Asp Trp Trp Ser Asn Phe Ser Thr Gln His Phe Arg Glu Gln	
635 640 645	
tca gag tgc atg atc tac cag tac ggc aac tac tcc tgg gac ctg gca	220
Ser Glu Cys Met Ile Tyr Gln Tyr Gly Asn Tyr Ser Trp Asp Leu Ala	
650 655 660	
gac gaa cag aac gtg aac gga ttc aac acc ctt ggg gaa aac att gct	224
Asp Glu Gln Asn Val Asn Gly Phe Asn Thr Leu Gly Glu Asn Ile Ala	
665 670 675	
gac aac gga ggg gtg cgg caa gcc tat aag gcc tac ctc aag tgg atg	229
Asp Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Lys Trp Met	
680 685 690 695	

Fig. 1C

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gca gag ggt ggc aag gac cag cag ctg ccc ggc ctg gat ctc acc cat	2344
Ala Glu Gly Gly Lys Asp Gln Gln Leu Pro Gly Leu Asp Leu Thr His	
700 705 710	
gag cag ctc ttc ttc atc aac tat gcc cag gtg tgg tgc ggg tcc tac	2391
Glu Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr	
715 720 725	
cgg ccc gag ttc gcc atc caa tcc atc aag aca gac gtc cac agt ccc	2441
Arg Pro Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro	
730 735 740	
ctg aag tac agg gta ctg ggg tgg ctg cag aac ctg gcc gcc ttc gca	2481
Leu Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Ala Ala Phe Ala	
745 750 755	
gac acg ttc cac tgt gcc cgg ggc acc ccc atg cac ccc aag gag cga	2531
Asp Thr Phe His Cys Ala Arg Gly Thr Pro Met His Pro Lys Glu Arg	
760 765 770 775	
STOP SEQ ID NO:3	
tgc cgc gtg tgg tagccaaggc cctgccgcgc tgtgcggccc acgcccacct	2581
Cys Arg Val Trp	
STOP SEQ ID NO:2	
gctgctcgga ggcattctgtg cgaagggtgca gctagcggcg acccagtgtg cgtccccgccc	2641
cggccaacca tgccaagcct gcctgccagg cctctgcgcc tggcctaggg tgcagccacc	2701
tgctgacac ccagggatga gcagtgtcca gtgcagtacc tggaccggag cccctccac	2761
agacacccgc ggggctcagt gccccgtca cagctctgta gagacaatca actgtgtcct	2821
gcccaccctc caagggtgcat tgtcttcag tatctacagc ttcagacttg agctaagtaa	2881
atgcttcaca gctatcacca tccttcctgt tttgaagcgt gaccgtggga tcctcaagag	2941
tcagc	2951
STOP SEQ ID NO:1	

Fig. 1D

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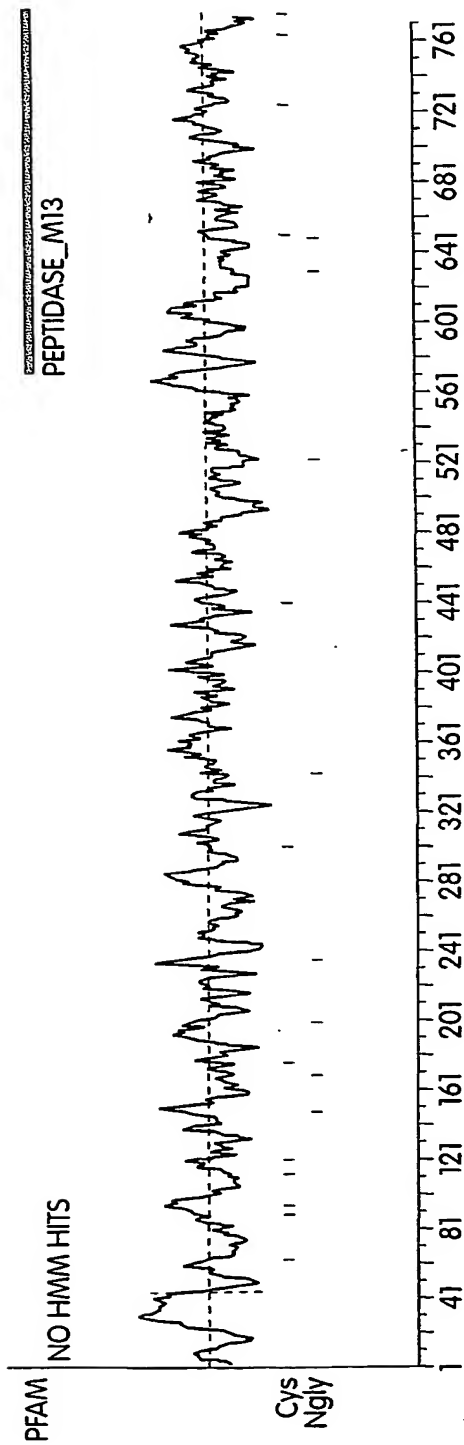


Fig. 2

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## Alignments of top-scoring domains:

Peptidase\_M13: domain 1 of 1, from 572 to 778: score 270.4, E = 2.4e-77

```

      *->nAyynpEfNsltfpagylPilvpPfddltyPravnskyGgiGfVlgH
      nA+y+p++N+++fpag  il+pPff  ++P+a n  +GgiG V+gH
Fbh56638FL  572  NAFYSPNRNQIVFPAG---ILQPPFFSKEQPQALN--FGGIGMVIGH 61

      EigHgFDdqGiqlddeeGsLadcwnlrdWkwtddednkcfqdrvkclidqys
      Ei+HgFDd+G+++d++G      n+ dW w + + + f+++++c+i qy
Fbh56638FL  614  EITHGFDDNGRNFDKNG-----NMMDW-WSNFSTQHFREQSECMIYQYG 61

      eavkPddkgwfnhcankGattlgEniADlgGlrAAlkaykkkssdeereE
      ++      +++ +++      G  tlgEniAD gG+r A+kay k +++ +
Fbh56638FL  657  NYSWDLADEQNVN----GFNTLGENIADNGGVRQAYKAYLKWMAEGG-KD 71

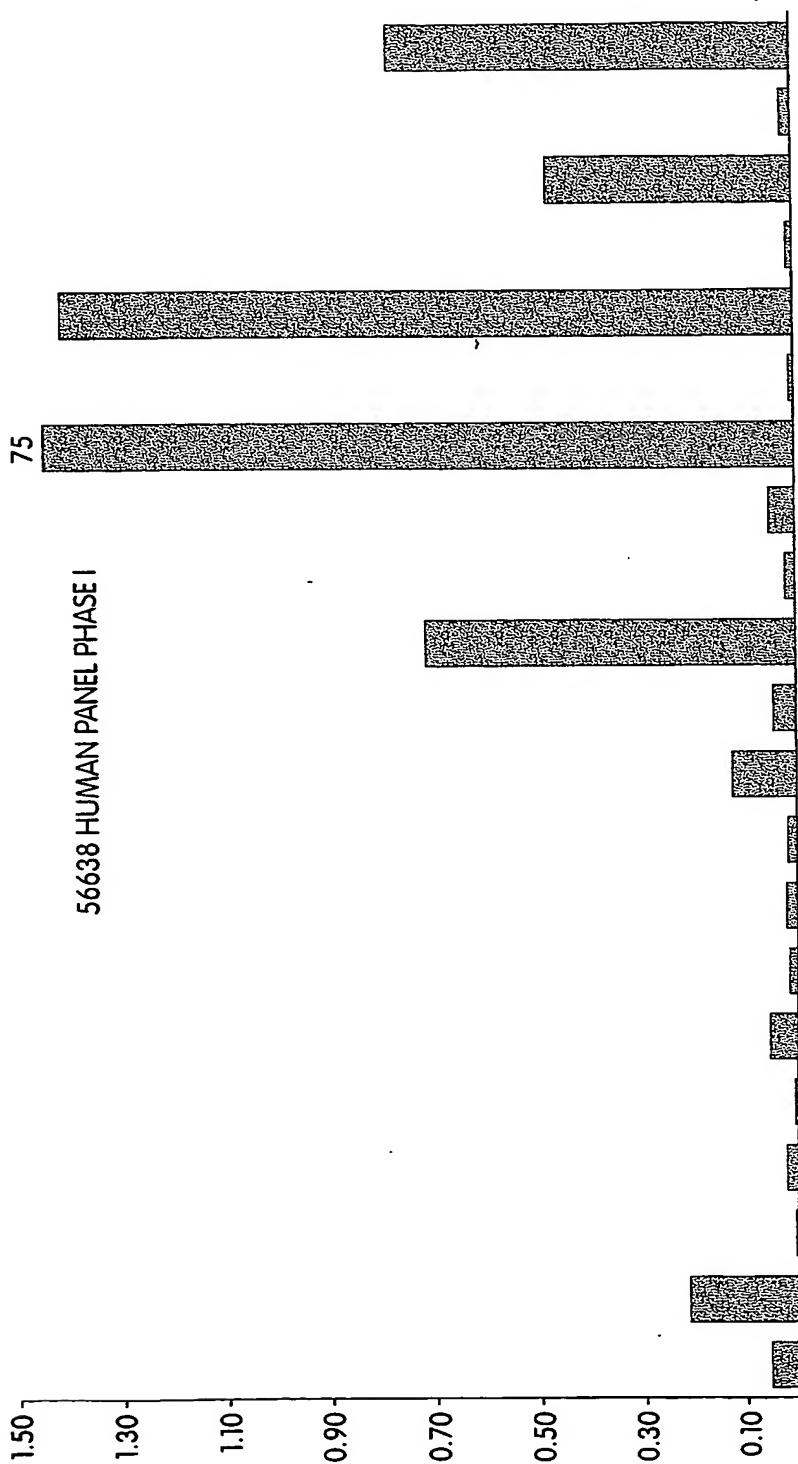
      qrlpgLenftpdQLfFetyAyswCrksrpenslrpllvdpHsPnelRvNg
      q+lpGL+ +t++QLfF++yA++wC  +rpe  ++ + +d+HsP+++Rv g
Fbh56638FL  702  QQLPGLD-LTHEQLFFINYAQVWCGRPEFAIQSIKTDVHSPLKYRVLG 71

      avrnmPaFasaFnCkpGdkMfpepekrc<-*
      ++n  aFa++F C++G +M p+ ++r+
Fbh56638FL  751  SLQNLAADFADTFHCARGTPMHPKERCRV      778

```

Fig. 3

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	ADRENAL GLAND	BRAIN	HEART	KIDNEY	LIVER	LUNG	MAMMARY GLAND	PLACENTA	PROSTATE	SALIVARY GLAND	MUSCLE	SM. INTESTINE	SPLEEN	STOMACH	TESTE	THYMUS	TRACHEA	UTERUS	SPINAL CORD	SKIN	DRG
56638 MEAN	35.18	34.42	39.41	35.60	38.93	33.00	35.62	35.70	35.83	33.72	37.22	30.68	34.11	34.43	25.42	37.66	30.47	37.35	31.80	34.70	31.40
82M803 MEAN	20.97	22.24	20.92	20.11	21.06	18.83	19.53	20.14	19.92	20.77	22.70	20.24	18.32	20.11	21.68	20.13	21.01	20.43	20.78	18.92	21.09
201	14.21	12.19	18.49	15.49	17.88	14.17	16.09	15.57	15.91	12.95	14.52	10.44	15.79	14.32	3.74	17.53	9.46	16.92	11.02	15.79	10.31
EXPRESSION	0.05	0.21	0.00	0.02	0.00	0.05	0.01	0.02	0.02	0.13	0.04	0.72	0.02	0.05	1.45	0.01	1.42	0.01	0.48	0.02	0.79